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(21) International Application Number: PCT/US98/12569 (22) International Filing Date: 17 June 1998 (17.06.98) (30) Priority Data: 60/050,962 18 June 1997 (18.06.97) US (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): KENDALL, Richard, L. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). THOMAS, Kenneth, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). MAO, Xianzhi [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). TEBBEN, Andrew [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: HUMAN RECEPTOR TYROSINE KINASE, KDR (57) Abstract An isolated nucleic acid molecule encoding a novel human receptor type tyrosine kinase gene, KDR, is disclosed. The isolation of this KDR cDNA sequence results in disclosure of purified forms of human KDR protein, recombinant vectors and recombinant hosts which express human KDR.		

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TITLE OF THE INVENTION

HUMAN RECEPTOR TYROSINE KINASE, KDR

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This non-provisional application is a continuation-in-part of U.S. Provisional Application Serial No. 60/050,962, filed June 18, 1997.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

10 Not applicable

REFERENCE TO MICROFICHE APPENDIX

Not applicable

15 FIELD OF THE INVENTION

The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes a human receptor tyrosine kinase, KDR, which is expressed on human endothelial cells. This receptor is activated by VEGF and mediates a mitogenic signal. The present invention also relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding human KDR, a DNA fragment encoding the intracellular portion of KDR, a DNA fragment encoding the extracellular portion of KDR with or without a membrane anchor sequence, substantially purified forms of associated human KDR, and human mutant forms of KDR.

BACKGROUND OF THE INVENTION

Vascular endothelial cells form a luminal non-thrombogenic monolayer throughout the vascular system. Mitogens promote embryonic vascular development, growth, repair and angiogenesis in these cells. Angiogenesis involves the proteolytic degradation of the basement membrane on which endothelial cells reside followed by the subsequent chemotactic migration and mitosis of these cells to support sustained growth of a new capillary shoot. One class of mitogens selective for vascular endothelial cells include vascular endothelial growth factor (referred to as VEGF or VEGF-A)

and the homologues placenta growth factor (PlGF), VEGF-B and VEGF-C.

Human VEGF exists as a glycosylated homodimer in one of four mature processed forms containing 206, 189 (see U.S. Patent No. 5,240,848), 165 (see U.S. Patent No. 5,332,671), and 121 (U.S. Patent No. 5,332,671) amino acids, the most prevalent being the 165 amino acid form. The 206 amino acid and 189 amino acid forms of human VEGF each contain a highly basic 24-amino acid insert that promotes tight binding to heparin, and presumably, heparin proteoglycans on cellular surfaces and within extracellular matrices (Ferrara et al., 1991, *J. Cell. Biochem.* 47: 211-218).

Human PlGF is also a glycosylated homodimer which shares 46% homology with VEGF at the protein level. Differential splicing of human PlGF mRNA leads to either a 170 or 149 amino acid residue precursor, which are proteolytically processed to mature forms of 152 or 131 amino acid residues in length, respectively (Maglione et al., 1993, *Oncogene* 8: 925-931; Bayne and Thomas, 1992, EPO Publication No. 0 506 477 A1; Hauser and Weich, 1993, *Growth Factors* 9: 259-268).

VEGF-B has been isolated and characterized (Grimmond et al., 1996, *Genome Research* 6: 124-131; Olofsson et al., 1996, *Proc. Natl. Acad. Sci. USA* 93: 2576-2581). The full-length human cDNAs encode 188 and 207 amino acid residue precursors wherein the NH₂ terminal portions are proteolytically processed to mature forms 167 and 186 amino acid residues in length. Human VEGF-B expression was found predominantly in heart and skeletal muscle as a disulfide-linked homodimer. However, human VEGF-B may also form a heterodimer with VEGF (*id.* @ 2580).

VEGF-C has also been isolated and characterized (Joukov et al., 1996, *EMBO J.* 15: 290-298). A cDNA encoding VEGF-C was obtained from a human prostatic adenocarcinoma cell line. A 32 kDa precursor protein is proteolytically processed to generate the mature 23 kDa form, which binds the receptor tyrosine kinase, Flt-4.

VEGF and its homologues impart activity by binding to vascular endothelial cell plasma membrane-spanning tyrosine kinase receptors which then activate an intracellular mitogenic signal. The

KDR receptor family is the major tyrosine kinase receptor which transduces the mitogenic signal initiated by VEGF.

Shibuya et al. (1990, *Oncogene* 5: 519-524) disclose a human receptor type tyrosine kinase gene *flt*, which comprises a 4.2 Kb open reading frame encoding a 1338 amino acid protein which comprises a glycosylated extracellular domain, membrane spanning region and predicted tyrosine kinase domain.

Pajusola et al. (1992, *Cancer Res.* 52: 5738-5743) disclose a human receptor type tyrosine kinase gene which, as noted above, binds human VEGF-C.

Vascular endothelial growth factor (VEGF) binds the high affinity membrane-spanning tyrosine kinase receptors KDR and Flt-1. Cell culture and gene knockout experiments indicate that each receptor contributes to different aspects of angiogenesis. KDR mediates the mitogenic function of VEGF whereas Flt-1 appears to modulate non-mitogenic functions such as those associated with cellular adhesion. Inhibiting KDR thus significantly diminishes the level of mitogenic VEGF activity.

Vascular growth in the retina leads to visual degeneration culminating in blindness. VEGF accounts for most of the angiogenic activity produced in or near the retina in diabetic retinopathy. Ocular VEGF mRNA and protein are elevated by conditions such as retinal vein occlusion in primates and decreased pO₂ levels in mice that lead to neovascularization. Intraocular injections of either anti-VEGF monoclonal antibodies or VEGF receptor immunofusions inhibit ocular neovascularization in rodent and primate models. Regardless of the cause of induction of VEGF in human diabetic retinopathy, inhibition of ocular VEGF is useful in treating the disease.

Expression of VEGF is also significantly increased in hypoxic regions of animal and human tumors adjacent to areas of necrosis. Monoclonal and polyclonal anti-VEGF antibodies inhibit the growth of human tumors in nude mice. Although these same tumor cells continue to express VEGF in culture, the antibodies do not diminish their mitotic rate of most, if not all, tumor cells derived from cells other than vascular endothelial cells themselves. Thus tumor-derived VEGF does not function as an autocrine mitogenic factor for

most tumors. Therefore, VEGF contributes to tumor growth *in vivo* by promoting angiogenesis through its paracrine vascular endothelial cell chemotactic and mitogenic activities. These monoclonal antibodies also inhibit the growth of typically less well vascularized human colon
5 cancers in athymic mice and decrease the number of tumors arising from inoculated cells. Viral expression of a VEGF-binding construct of Flk-1, the mouse KDR receptor homologue, truncated to eliminate the cytoplasmic tyrosine kinase domains but retaining a membrane anchor, virtually abolishes the growth of a transplantable glioblastoma in mice
10 presumably by the dominant negative mechanism of heterodimer formation with membrane-spanning endothelial cell VEGF receptors. Embryonic stem cells, which normally grow as solid tumors in nude mice, do not produce detectable tumors if both VEGF alleles are knocked out. Taken together, these data indicate the role of VEGF in the growth
15 of solid tumors. KDR and Flt-1 are implicated in pathological neoangiogenesis, and inhibitors of these receptors are useful in the treatment of diseases in which neoangiogenesis is part of the overall pathology, e.g., diabetic retinal vascularization, various forms of cancer as well as forms of inflammation such as rheumatoid arthritis,
20 psoriasis, contact dermatitis and hypersensitivity reaction.

Terman et al. (1991, *Oncogene* 6: 1677-1683; 1992, *Biochem. Biophys. Res. Commun.* 187: 1579-1586) disclose a full-length cDNA encoding a form of KDR. However, the Terman et al. disclosures do not identify a novel, optimal nucleic acid fragment encoding the human
25 form of the receptor type tyrosine kinase gene, KDR. It will be advantageous to identify and isolate a human cDNA sequence encoding an optimized form of human KDR. A nucleic acid molecule expressing the human KDR protein will be useful in screening for compounds acting as a modulator of the protein kinase domain of this protein. Such
30 a compound or compounds will be useful in modulating the mitogenic signal of VEGF and VEGF-related proteins on vascular endothelial cells. The KDR nucleic acid sequence may be also useful for gene therapy encoding a portion of the KDR protein that would contain functional ligand binding and membrane anchoring moieties but not
35 tyrosine kinase activity. Either all or a portion of the KDR protein is also useful to screen for VEGF antagonists. The KDR nucleic acid sequence

can be transfected into cells for analysis of function in the absence of Flt-1. The KDR protein is also useful for x-ray structure analysis in the presence or absence of ligand and/or inhibitors. The present invention addresses and meets these needs by disclosing an isolated nucleic acid
5 fragment which expresses a form of human KDR which is shown by computer modeling to be predictive of higher activity and functionality than the previously disclosed KDR.

SUMMARY OF THE INVENTION

10 The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes a novel human receptor type tyrosine kinase gene, KDR. This specification discloses a novel, optimized DNA molecule which encodes, KDR, a receptor tyrosine kinase expressed on human endothelial cells.

15 The present invention also relates to biologically active fragments or mutants of SEQ ID NO:1 which encodes mRNA expressing a novel human receptor type tyrosine kinase gene, KDR. Any such biologically active fragment and/or mutant will encode either a protein or protein fragment comprising at least an intracellular or extracellular
20 kinase domain similar to that of the human KDR protein as set forth in SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of
25 diagnostic, therapeutic or prophylactic use and would be useful for screening for agonists and/or antagonists for KDR function.

The isolated nucleic acid molecule of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary DNA (cDNA), which may be single (coding or
30 noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

35 The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain

the substantially purified nucleic acid molecules disclosed throughout this specification.

5 The present invention also relates to subcellular membrane fractions of the recombinant host cells (both prokaryotic and eukaryotic as well as both stably and transiently transformed cells) comprising the nucleic acids of the present invention. These subcellular membrane fractions will comprise either wild-type or human mutant forms of KDR at levels substantially above wild-type levels and hence will be useful in various assays described throughout this specification.

10 A preferred aspect of the present invention is disclosed in Figure 1A and Figure 1B and SEQ ID NO:1, a human cDNA encoding a novel receptor type tyrosine kinase gene, KDR.

15 The present invention also relates to a substantially purified form of the receptor type tyrosine kinase gene, KDR which is disclosed in Figure 2 and as set forth in SEQ ID NO:2.

20 The present invention also relates to biologically active fragments and/or mutants of the KDR protein as initially set forth as SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or prophylactic use and would be useful for screening for agonists and/or antagonists for KDR function.

25 A preferred aspect of the present invention is disclosed in Figure 2 and is set forth as SEQ ID NO:2, the amino acid sequence of the novel receptor type tyrosine kinase gene, KDR.

The present invention also relates to polyclonal and monoclonal antibodies raised in response to either the human form of KDR disclosed herein, or a biologically active fragment thereof.

30 The present invention also relates to isolated nucleic acid molecules which are fusion constructions expressing fusion proteins useful in assays to identify compounds which modulate wild-type human KDR activity. A preferred aspect of this portion of the invention includes, but is not limited to, glutathione S-transferase (GST)-KDR fusion constructs. These fusion constructs include, but are not limited to, either the intracellular tyrosine kinase domain of human KDR as an

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in-frame fusion at the carboxy terminus of the GST gene or the extracellular ligand binding domain fused to an immunoglobulin gene by methods known to one of ordinary skill in the art. Soluble recombinant GST-kinase domain fusion proteins may be expressed in
5 various expression systems, including *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) using a baculovirus expression vector (pAcG2T, Pharmingen).

The present invention also relates to isolated nucleic acid molecules which encode human KDR protein fragments comprising a
10 portion of the intracellular KDR domain. The protein fragments are useful in assays to identify compounds which modulate wild-type human KDR activity. A preferred aspect of this portion of the invention includes, but is not limited to, a nucleic acid construction which encodes the intracellular portion of human KDR, from about amino acid 780 - 795
15 to about amino acid 1175 - 1386.

Therefore, the present invention relates to isolated nucleic acid molecules which encode human KDR protein fragments comprising a portion of the extracellular KDR domain. These isolated nucleic acid proteins may or may not include nucleotide sequences
20 which also encode the transmembrane domain of human KDR. These KDR extracellular and/or KDR extracellular-transmembrane domain protein fragments will be useful in screening for compounds which inhibit VEGF binding as well as utilizing these isolated nucleic acids as gene therapy vehicles to inhibit VEGF-mediated mitogenic activity.
25 Expression of either a soluble version of KDR (extracellular) or membrane bound form (extracellular-transmembrane) will inhibit *in vivo* VEGF/KDR mediated angiogenesis.

Therefore, the present invention relates to methods of expressing the receptor type tyrosine kinase gene, KDR, and biological
30 equivalents disclosed herein, assays employing these receptor type tyrosine kinase genes, cells expressing these receptor type tyrosine kinase genes, and compounds identified through the use of these receptor type tyrosine kinase genes and expressed human KDR protein, including one or more modulators of the human KDR-dependent kinase
35 either through direct contact with the kinase domain of human KDR or a compound which prevents binding of VEGF to human KDR, or

appropriate dimerization of the KDR receptor antagonizing transduction of the normal intracellular signals associated with VEGF-induced angiogenesis.

5 The present invention also relates to gene therapy applications, especially for nucleic acid fragments which encode soluble extracellular protein fragments of human KDR. It is disclosed herein that such methods will be useful especially in the treatment of various tumors as well as diabetic retinopathy.

10 It is an object of the present invention to provide an isolated nucleic acid molecule which encodes a novel form of human KDR, or human KDR fragments and KDR mutants which are derivatives of SEQ ID NO:2 and preferably retain Val at position 848, and especially preferable is retention of Val at position 848, Glu at position 498, Ala at position 772, Arg at position 787, Lys at position 835 and Ser at position 15 1347. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of diagnostic, therapeutic or prophylactic use and would be useful for 20 screening for agonists and/or antagonists for KDR function.

It is a further object of the present invention to provide the human KDR proteins or protein fragments encoded by the nucleic acid molecules referred to in the preceding paragraph.

25 It is also an object of the present invention to provide biologically active fragments or mutants of human KDR which comprise an intracellular kinase domain similar to that of the human KDR protein as set forth in SEQ ID NO:2, preferably retaining Val at position 848, and especially preferable is retention of Val at position 848, Glu at position 498, Ala at position 772, Arg at position 787, Lys at position 835 30 and Ser at position 1347.

It is a further object of the present invention to provide recombinant vectors and recombinant host cells which comprise a nucleic acid sequence encoding human KDR or a biological equivalent thereof.

It is an object of the present invention to provide a substantially purified form of the receptor type tyrosine kinase gene, KDR, as set forth in SEQ ID NO:2.

It is an object of the present invention to provide for
5 biologically active fragments and/or mutants of the KDR protein, such as set forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic,
10 therapeutic or prophylactic use.

It is also an object of the present invention to provide for KDR-based in-frame fusion constructions, methods of expressing the receptor type tyrosine kinase gene, KDR, and biological equivalents disclosed herein, related assays, recombinant cells expressing these
15 receptor type tyrosine kinase genes, and agonistic and/or antagonistic compounds identified through the use of these receptor type tyrosine kinase genes and expressed human KDR protein.

As used herein, "VEGF" or "VEFG-A" refers to vascular endothelial growth factor.

20 As used herein, "KDR" or "FLK-1" refers to kinase insert domain-containing receptor.

As used herein, "FLT-1" refers to fms-like tyrosine kinase receptor.

25 As used herein, the term "mammalian host" refers to any mammal, including a human being.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A and Figure 1B show the nucleotide sequence which encodes human KDR, as set forth in SEQ ID NO:1.

30 Figure 2 shows the amino acid sequence of human KDR, as also set forth in SEQ ID NO:2. Underlined amino acid residues represent differences in comparison to a previously disclosed form of human KDR.

Figure 3A shows the ATP binding domain from the KDR V848E mutant homology model with bound AMP-PCP. The side chain of
35 E848 is in contact the adenine from AMP-PCP. The gamma phosphate

of AMP-PCP is not visible. The protein carbon alpha trace is shown in pipes, the AMP-PCP in sticks and the E848 side chain in space filling. The N-terminal lobe is colored blue (or alternatively labeled with light circles) with the exception of the glycine rich flap which is colored green (or alternatively labeled as a lined region). The C-terminal lobe is colored red (or alternatively labeled with dark circles).

Figure 3B shows ATP binding domain from the KDR homology model with bound AMP-PCP. The side chain of V848 forms hydrophobic contacts with the adenine from AMP-PCP. The gamma phosphate of AMP-PCP is not visible. The protein carbon alpha trace is shown in pipes, the AMP-PCP in sticks and the V848 side chain in space filling. The N-terminal lobe is colored blue (or alternatively labeled with light circles) with the exception of the glycine rich flap which is colored green (or alternatively labeled as a lined region). The C-terminal lobe is colored red (or alternatively labeled with dark circles).

Figure 4A and 4B show that purified GST-KDR_{cyt}E848 was unable to autophosphorylate in the presence of 1-mM ATP wherein 12 ng of GST-KDR_{cyt}V848 in the presence of 1 mM ATP resulted in autophosphorylation (Figure 4A) and that both both 120 ng of GST-KDR_{cyt}E848 and 12 ng of GST-KDR_{cyt}V848 react with anti-KDR antibody (Figure 4B).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to isolated nucleic acid and protein forms which represent human KDR. This specification discloses a DNA molecule encoding human KDR, a receptor tyrosine kinase expressed on human endothelial cells. The receptor is activated by vascular endothelial growth factor (VEGF) and mediates a mitogenic signal. This activation and subsequent mitogenesis leads to an angiogenic response *in vivo*. The nucleic acid molecule disclosed in the specification as SEQ ID NO:1 encodes a human KDR protein (SEQ ID NO:2) which results in six amino acid differences from the published sequence (Terman et al., 1992, *Biochem. Biophys. Res. Commun.* 187: 1579-1586, Terman et al., International PCT application number WO 92/14748, International application number PCT/US92/01300). These changes are position 498 (Ala to Glu), 772 (Thr to Ala), 787 (Gly to Arg),

835 (Asn to Lys), 848 (Glu to Val), and 1347 (Thr to Ser). These six amino acid changes affect the activity of the receptor. Val 848 is conserved throughout most of the tyrosine kinase family and appears to be important for the binding of ATP and presumably ATP competitive inhibitors to the KDR receptor kinase as inferred by computer modeling. A change to Glu at this position results in a non-functional kinase as a consequence of impaired ATP binding. The other changes may also cause activity differences.

The present invention also relates to either biologically active fragments or mutants of SEQ ID NO:1 which encodes mRNA expressing a novel human receptor type tyrosine kinase gene, KDR. Any such biologically active fragment and/or mutant will encode a protein or protein fragment comprising at least an intracellular kinase domain similar to that of the human KDR protein as set forth in SEQ ID NO:2 and preferably retain Val at position 848. It is also envisioned that other intracellular-based KDR domains will result in a soluble protein fragment which mimics wild-type intracellular domain structure and function. Any such protein fragment may be a fusion protein, such as the exemplified GST-KDR fusion, or may be solely comprised of the KDR intracellular domain, with increasing deletions in from the COOH-terminal region. It is especially preferable that the following amino acids be retained, if this domain encompasses the respective protein or protein fragment: Val at position 848, Glu at position 498, Ala at position 772, Arg at position 787, Lys at position 835 and Ser at position 1347. Therefore, any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of diagnostic, therapeutic or prophylactic use and is useful for the identification of modulators of KDR receptor activity.

The isolated nucleic acid molecule of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic

acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

It is known that DNA sequences coding for a peptide may be altered so as to code for a peptide having properties that are different
5 than those of the naturally occurring peptide. Methods of altering the DNA sequences include but are not limited to site directed mutagenesis. Examples of altered properties include but are not limited to changes in the affinity of an enzyme for a substrate or a receptor for a ligand.

As used herein, "purified" and "isolated" are utilized
10 interchangeably to stand for the proposition that the nucleic acid, protein, or respective fragment thereof in question has been substantially removed from its *in vivo* environment so that it may be manipulated by the skilled artisan, such as but not limited to nucleotide sequencing, restriction digestion, site-directed mutagenesis, and
15 subcloning into expression vectors for a nucleic acid fragment as well as obtaining the protein or protein fragment in pure quantities so as to afford the opportunity to generate polyclonal antibodies, monoclonal antibodies, amino acid sequencing, and peptide digestion. Therefore, the nucleic acids claimed herein may be present in whole cells or in cell
20 lysates or in a partially purified or substantially purified form. A nucleic acid is considered substantially purified when it is purified away from environmental contaminants. Thus, a nucleic acid sequence isolated from cells is considered to be substantially purified when purified from cellular components by standard methods while a
25 chemically synthesized nucleic acid sequence is considered to be substantially purified when purified from its chemical precursors.

The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout
30 this specification.

The present invention also relates to subcellular membrane fractions of the recombinant host cells (both prokaryotic and eukaryotic as well as both stably and transiently transformed cells) comprising the nucleic acids of the present invention. These subcellular membrane
35 fractions will comprise wild-type or human mutant forms of KDR at

levels substantially above wild-type levels and hence will be useful in various assays described throughout this specification.

A preferred aspect of the present invention is disclosed in Figure 1A and Figure 1B and SEQ ID NO:1, a human cDNA encoding a novel receptor type tyrosine kinase gene, KDR, disclosed as follows:

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ATGGAGAGCAAGGTGCTGCTGGCCGTCGCCCTGTGGCTCTGCGTGGAGACCCGGGCGCCTCTGTGGGT
TTGCCTAGTGTCTTCTTGGATCTGCCCAGGCTCAGCATACAAAAGACATACTTACAATTAAGGCTAAT
ACAACCTCTTCAAATTACTTGCAGGGGACAGAGGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGC
10 AGTGAGCAAAGGGTGGAGGTGACTGAGTGCAGCGATGGCCTCTTCTGTAAGACACTCACAATTCCAAAA
GTGATCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGGAACCTGACTTGGCCTCGGTCATTAT
GTCTATGTTCAAGATTACAGATCTCCATTTATTGCTTCTGTTAGTGACCAACATGGAGTCGTGTACATT
ACTGAGAACAAAAACAAAACCTGTGGTGATTCCATGTCTCGGGTCCATTTCAAATCTCAACGTGTCACTT
TGTGCAAGATACCCAGAAAAGAGATTTGTTCCCTGATGGTAACAGAATTTCCCTGGGACAGCAAGAAGGGC
15 TTTACTATTCCCAGCTACATGATCAGCTATGCTGGCATGGTCTTCTGTGAAGCAAAAATTAATGATGAA
AGTTACCAGTCTATTATGTACATAGTTGTCTGTTAGGGTATAGGATTTATGATGTGGTTCTGAGTCCG
TCTCATGGAATTGAACATCTGTTGGAGAAAAGCTTGTCTTAAATTGTACAGCAAGAAGTGAACATAAAT
GTGGGGATTGACTTCAACTGGGAATACCCCTTCTTCGAAGCATCAGCATAAGAACTTGTAACCCGAGAC
CTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACATATAGATGGTGTAACCCGG
20 AGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTTGTC
AGGGTCCATGAAAAACCTTTTGTGCTTTTGGAAAGTGGCATGGAATCTCTGGTGGAAAGCCACGGTGGGG
GAGCGTGTGAGAATCCCTGCGAAGTACCTTGGTTACCCACCCCGAGAAATAAAATGGTATAAAAATGGA
ATACCCCTTGAGTCCAATCACACAATTAAGCGGGGCATGTACTGACGATTATGGAAGTGAGTGAAAGA
GACACAGGAAATTACACTGTCTATCCTTACCAATCCCATTTCAAAGGAGAAGCAGAGCCATGTGGTCTCT
25 CTGGTTGTGTATGTCACCCACCCAGATTGGTGAGAAATCTTAATCTCTCCTGTGGATTCTTACCAGTAC
GGCACCACTCAAACGCTGACATGTACGGTCTATGCCATTCTCCCTCCCGCATCACATCCACTGATTGG
CAGTTGGAGGAAGAGTGCGCCAACGAGCCAGCCAAGCTGTCTCAGTGACAAACCCATACCCTTGTGAA
GAATGGAGAAGTGTGGAGGACTTCCAGGGAGGAAATAAAATGAAGTTAATAAAAATCAATTTGCTCTA
ATTGAAGGAAAAACAAAACCTGTAAGTACCCTTGTATCCAAGCGGCAAATGTGTGAGCTTTGTACAAA
30 TGTGAAGCGGTCAACAAAGTCGGGAGAGGAGAGAGGGTGATCTCCTTCCACGTGACCAGGGGTCTTGAA
ATTACTTTTGCAACCTGACATGCAGCCCACTGAGCAGGAGAGCGTGTCTTTGTGGTGCAGTGCAGACAGA
TCTACGTTTGTGAGAACCCTCACATGGTACCAAGCTTGGCCCCACAGCCTCTGCCAATCCATGTGGGAGAGTTG
CCCACACCTGTTTGAAGAAGTGGATACTCTTTGGAAATTGAATGCCACCATTGTCTCTGACACACA
AATGACATTTTGTATCATGGAGCTTAAGAATGCATCCTTGCAGGACCAAGGAGACTATGTCTGCCTTGCT
35 CAAGACAGGAAGACCAAGAAAAGACATTGCGTGGTCAGGCAGCTCACAGTCTTAGAGCGTGTGGCAGCC
ACGATCACAGGAAACCTGGAGAATCAGACGACAAGTATTGGGGAAAGCATCGAAGTCTCATGCACGGCA
TCTGGGAATCCCCCTCCACAGATCATGTGGTTTAAAGATAATGAGACCCCTGTAGAAAGACTCAGGCATT
GTATTGAAGGATGGGAACCGGAACCTCACTATCCGAGAGTGAGGAAGGAGGACGAAGGCCTCTACACC
TGCCAGGCATGCAGTGTCTTGGCTGTGCAAAAGTGGAGGCATTTTTCATAATAGAAGGTGCCAGGAA
40 AAGACGAACTTGGAAATCATTTATTCTAGTAGGCACGGCGGTGATTGCCATGTTCTTCTGGCTACTTCTT
GTCATCATCCTACGGACCGTTAAGCGGGCCAATGGAGGGGAAGTGAAGACAGGCTACTTGTCCATCGTC
ATGGATCCAGATGAACCTCCCATTTGGATGAACATTGTGAACGACTGCCTTATGATGCCAGCAAATGGGAA
TTCCCCAGAGACCGGCTGAAGCTAGGTAAGCCTCTTGGCCGTGGTGCCTTTGGCCAAGTGATTGAAGCA
GATGCCTTTTGGAAATTGACAAGACAGCAACTTGCAGGACAGTAGCAGTCAAAATGTTGAAAGAAGGAGCA
45 ACACACAGTGAGCATCGAGCTCTCATGTCTGAACTCAAGATCCTCATTCATATTGGTCAACCATCTCAAT
GTGGTCAACCTTCTAGGTGCCTGTACCAAGCCAGGAGGGCCACTCATGGTGATTGTGGAATTTCTGCAAA
TTTGGAAACCTGTCCACTTACCTGAGGAGCAAGAGAAATGAATTTGTCCCTACAAGACCAAGGGGCA
CGATTCCGTCAGGGAAGACTACGTTGGAGCAATCCCTGTGGATCTGAAACGGCGCTTGGACAGCATC
ACCAGTAGCCAGAGCTCAGCCAGCTCTGGATTTGTGGAGGAGAAGTCCCTCAGTGATGTAGAAGAAGAG
50 GAAGCTCCTGAAGATCTGTATAAGGACTTCCTGACCTTGGAGCATCTCATCTGTTACAGCTTCCAAGTG
GCTAAGGCGATGGAGTTCTTGGCATCGCGAAAGTGATCCACAGGGACCTGGCGGCACGAAATATCCTC
TTATCGGAGAAGAAGCTGGTTAAATCTGTGACTTTGGCTTGGCCCGGGATATTTATAAAGATCCAGAT
TATGTCAGAAAAGGAGATGCTCGCCTCCCTTTGAAATGGATGGCCCCAGAAACAATTTTGTACAGAGTG
TACACAATCCAGAGTGACGTCTGGTCTTTTGGTGTGCTGTGGGAAATATTTTCCCTTAGGTGCTTCT
55 CCATATCCTGGGGTAAAGATTGATGAAGAATTTTGTAGGCGATTGAAAGAAGGAAC TAGAATGAGGGCC

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CCTGATTATACTACACCAGAAATGTACCAGACCATGCTGGACTGCTGGCACGGGGAGCCCAGTCAGAGA
 CCCACGTTTTTCAGAGTTGGTGGAACATTTGGGAAATCTCTTGCAAGCTAATGCTCAGCAGGATGGCAAA
 GACTACATTGTTCTTCCGATATCAGAGACTTTGAGCATGGAAGAGGATTCTGGACTCTCTCTGCCTACC
 TCACCTGTTTCTGTATGGAGGAGGAGGAAGTATGTGACCCCAAATTCCATTATGACAACACAGCAGGA
 5 ATCAGTCAGTATCTGCAGAACAGTAAGCGAAAAGAGCCCGCCTGTGAGTGTAAAAACATTTGAAGATATC
 CCGTTAGAAGAACCAGAAGTAAAAGTAATCCAGATGACAACCAGACGGACAGTGGTATGGTTCTTGCC
 TCAGAAGAGCTGAAAACATTTGGAAGACAGAACCAAATTATCTCCATCTTTTGGTGGAAATGGTGGCCAGC
 AAAAGCAGGGAGTCTGTGGCATCTGAAGGCTCAAACCAGACAAGCGGCTACCAGTCCGGATATCACTCC
 10 GATGACACAGACACCACCGTGTACTCCAGTGAGGAAGCAGAACTTTTAAAGCTGATAGAGATTGGAGTG
 CAAACCGGTAGCACAGCCCAGATTCTCCAGCCTGACTCGGGGACCACACTGAGCTCTCCTCTGTTTAA
 (SEQ ID NO:1).

The present invention also relates to a substantially purified
 form of the receptor type tyrosine kinase gene which comprises the KDR
 15 amino acid sequence disclosed in Figure 2 and as set forth in SEQ ID
 NO:2, which includes Glu at position 498, Ala at position 772, Arg at
 position 787, Lys at position 835, Val at position 848 and Ser at position
 1347, disclosed as follows:

20 MESKVLVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLWLWPNNQSG
 SEQRVEVTECSDFLCKTLTIKVIIGNDTGAYKCFYRETDLASVIYVYVQDYRSPFIASVSDQHGVVYI
 TENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVFEAKINDE
 SYQSIMYIVVVVGRIYDVVLSPSHGIELSVGEKLVNCTARTELNVGIDFNWEYPSSKHQHKLVNRD
 25 LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATVG
 ERVRIPAKYLGYPPEIKWYKNGIPLSNHTIKAGHVLTIMEVSEKDTGNYTVILTNPISKEKQSHVVS
 LVVYVPPQIGEKSLISPVDSYQYGTQTTLCTVYAIPPPHHIHWYQLEEECANEPSQAVSVTNPYPCPE
 EWRSVEDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTRGPE
 ITLQPDMPTEQESVSLWCTADRSTFENLTWYKLGQPLPIHVGEPLTPVCKNLDTLWKLNATMFSNST
 30 NDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVRQLTVLERVAPTITGNLENQTTSIGESIEVSCSTA
 SGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIIRVRKEDEGLYTCQACSVLGCARKEAFFIIEGAQE
 KTNLEIIILVGTAVIAMFFWLLLVIILRTVTKRANGGELKTGYLSIVMDPDELPLDEHCEKRLPYDASKWE
 FPRDRLKLGKPLGRGAFGQVIEADAFGIDKTATCRTVAVKMLKEGATHSEHRAIMSELKILIHIGHHLN
 VVNLGACTKPGGPLMVIVEFCKFGNLSTYLSKRNEFVYPYKTKGARFRQKDYVGAIPVDLKRRLDSI
 35 TSSQSSASSGFVEEKSLSVDEEEEAPELDYKDFLTLEHLICYSFQVAKGMEFLASRKCIIHRDLAARNIL
 LSEKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWMAPETIFDRVYTIQSDVWSFGVLLWEIFSLGAS
 PYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTMLDCWHGEPSQRPTFSELVEHLGNLLQANAQQDQK
 DYIVLPISETLSMEEDSGLSLPTSPVSCMEEEVCDPKFHYDNTAGISQYLQNSKRKSRPVSVKTFEDI
 PLEEPEVKVIPDDNQTDSGMVLASEELKTLEDRTKLSPSFSGMVPSKSRSESVASEGNSQTSQYQSGYHS
 DDTDTTVYSSEEAEELLKLEIGVQTGSTAQILQPDGTTLSPPV (SEQ ID NO:2).

40 The present invention also relates to biologically active
 fragments and/or mutants of the KDR protein as initially set forth as
 SEQ ID NO:2, including but not necessarily limited to amino acid
 substitutions, deletions, additions, amino terminal truncations and
 45 carboxy-terminal truncations such that these mutations provide for
 proteins or protein fragments of diagnostic, therapeutic or prophylactic
 use and would be useful for screening for agonists and/or antagonists
 for KDR function.

A preferred aspect of the present invention is disclosed in Figure 2 and is set forth as SEQ ID NO:2, the amino acid sequence of the novel receptor type tyrosine kinase gene, KDR.

5 The present invention also relates to isolated nucleic acid molecules which are fusion constructions useful in assays to identify compounds which modulate wild-type human KDR activity. A preferred aspect of this portion of the invention includes, but is not limited to, GST-KDR fusion constructs. These fusion constructs comprise the intracellular tyrosine kinase domain of human KDR as an in-frame
10 fusion at the carboxy terminus of the GST gene. Soluble recombinant GST-kinase domain fusion proteins may be expressed in various expression systems, including *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) using a baculovirus expression vector (pAcG2T, Pharmingen).

15 The present invention relates to isolated nucleic acid molecules which encode soluble portions of the KDR intracellular or extracellular domain. Especially preferred are nucleic acid molecules which encode a COOH-terminal deletion KDR protein fragment useful in assays to identify compounds which modulate wild-type human KDR
20 activity. Any such nucleic acid will encode a KDR protein fragment which mimics KDR wild-type activity within the respective domain, such as the kinase domain of human KDR. These expressed soluble protein fragments may or may not contain a portion of the amino-terminal region of human KDR or of a heterologous sequence. These
25 nucleic acids may be expressed in any of a number of expression systems available to the artisan. Any such intracellular-based KDR construction of the present invention may be utilized in gene therapy applications, such as acting as an soluble agonist or antagonist of kinase activity normally associated with wild type, membrane
30 associated kinase activity.

Therefore, the present invention relates to isolated nucleic acid molecules which encode human KDR protein fragments comprising a portion of the intracellular KDR domain. The protein fragments are useful in assays to identify compounds which modulate
35 wild-type human KDR activity. A preferred aspect of this portion of the invention includes, but is not limited to, a nucleic acid construction

which encodes the intracellular portion of human KDR, from about amino acid 780 - 795 to about amino acid 1175 - 1386. The data exemplified in Example Section 3 show that COOH terminal deletions of the soluble intracellular portion of KDR exhibit kinase activity.

5 The present invention also relates to isolated nucleic acid molecules which encode human KDR protein fragments comprising a portion of the extracellular KDR domain. These isolated nucleic acid proteins may or may not include nucleotide sequences which also encode the transmembrane domain of human KDR. These KDR
10 extracellular and/or KDR extracellular-transmembrane domain protein fragments will be useful in screening for compounds which inhibit VEGF binding as well as utilizing these isolated nucleic acids as gene therapy vehicles to inhibit VEGF-mediated mitogenic activity. Expression of wither a soluble version of KDR (extracellular) or
15 membrane bound form (extracellular-transmembrane) will inhibit VEGF/KDR mediated angiogenesis. A preferred aspect of this portion of the invention includes, but is not limited to, an isolated nucleic acid molecule which encodes at least six of the IG-like extracellular domains from the amino-terminal end of KDR. Such a protein fragment would
20 comprise at least from about the initiating methionine to about amino acid 644 of human KDR (SEQ ID NO:2). Another preferred aspect of this portion of the invention includes, but is not limited to, an isolated nucleic acid molecule which encodes the all seven IG-like extracellular domains from the amino-terminal end of KDR. Such a protein fragment
25 would comprise at least from about the initiating methionine to about amino acid 763. An additional preferred embodiment includes but is not limited to an extracellular-transmembrane construct which encodes about the initial 785 - 795 amino acids of KDR as set forth in SEQ ID NO:2, and especially preferred is an isolated nucleic acid molecule
30 construction which encodes the amino terminal portion of KDR with a truncation at about amino acid 791 as set forth in SEQ ID NO:2.

Therefore, the present invention relates to methods of expressing the receptor type tyrosine kinase gene, KDR, and biological equivalents disclosed herein, assays employing these receptor type
35 tyrosine kinase genes, cells expressing these receptor type tyrosine kinase genes, and agonistic and/or antagonistic compounds identified

through the use of these receptor type tyrosine kinase genes and expressed human KDR protein, including, but not limited to, one or more modulators of the human KDR-dependent kinase either through direct contact with the kinase domain of human KDR or a compound
5 which prevents binding of VEGF to human KDR, or either prevents or promotes receptor dimerization and/or activation thereby either inducing or antagonizing transduction of the normal intracellular signals associated with VEGF-induced angiogenesis

As used herein, a "biologically active equivalent" or
10 "functional derivative" of a wild-type human KDR possesses a biological activity that is substantially similar to the biological activity of the wild type human KDR. The term "functional derivative" is intended to include the "fragments," "mutants," "variants," "degenerate variants," "analogs" and "homologues" or to "chemical derivatives" of the wild type
15 human KDR protein. The term "fragment" is meant to refer to any polypeptide subset of wild-type human KDR. The term "mutant" is meant to refer to a molecule that may be substantially similar to the wild-type form but possesses distinguishing biological characteristics. Such altered characteristics include but are in no way limited to altered
20 substrate binding, altered substrate affinity and altered sensitivity to chemical compounds affecting biological activity of the human KDR or human KDR functional derivative. The term "variant" is meant to refer to a molecule substantially similar in structure and function to either the entire wild-type protein or to a fragment thereof. A molecule is
25 "substantially similar" to a wild-type human KDR-like protein if both molecules have substantially similar structures or if both molecules possess similar biological activity. Therefore, if the two molecules possess substantially similar activity, they are considered to be variants even if the structure of one of the molecules is not found in the other or
30 even if the two amino acid sequences are not identical. The term "analog" refers to a molecule substantially similar in function to either the full-length human KDR protein or to a biologically active fragment thereof.

Any of a variety of procedures may be used to clone human
35 KDR. These methods include, but are not limited to, (1) a RACE PCR cloning technique (Frohman, et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:

8998-9002). 5' and/or 3' RACE may be performed to generate a full-length cDNA sequence. This strategy involves using gene-specific oligonucleotide primers for PCR amplification of human KDR cDNA. These gene-specific primers are designed through identification of an expressed sequence tag (EST) nucleotide sequence which has been identified by searching any number of publicly available nucleic acid and protein databases; (2) direct functional expression of the human KDR cDNA following the construction of a human KDR-containing cDNA library in an appropriate expression vector system; (3) screening a human KDR-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled degenerate oligonucleotide probe designed from the amino acid sequence of the human KDR protein; and (4) screening a human KDR-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding the human KDR protein. This partial cDNA is obtained by the specific PCR amplification of human KDR DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence known for other kinases which are related to the human KDR protein; (5) screening a human KDR-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding the human KDR protein. This strategy may also involve using gene-specific oligonucleotide primers for PCR amplification of human KDR cDNA identified as an EST as described above; or (6) designing 5' and 3' gene specific oligonucleotides using SEQ ID NO: 1 as a template so that either the full-length cDNA may be generated by known RACE techniques, or a portion of the coding region may be generated by these same known RACE techniques to generate and isolate a portion of the coding region to use as a probe to screen one of numerous types of cDNA and/or genomic libraries in order to isolate a full-length version of the nucleotide sequence encoding human KDR.

It is readily apparent to those skilled in the art that other types of libraries, as well as libraries constructed from other cell types or species types, may be useful for isolating a human KDR-encoding DNA or a human KDR homologue. Other types of libraries include, but are not limited to, cDNA libraries derived from other cells or cell lines other than human cells or tissue such as murine cells, rodent cells or any

other such vertebrate host which may contain human KDR-encoding DNA. Additionally a human KDR gene and homologues may be isolated by oligonucleotide- or polynucleotide-based hybridization screening of a vertebrate genomic library, including but not limited to, a murine
5 genomic library, a rodent genomic library, as well as concomitant human genomic DNA libraries.

It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have KDR activity. The selection of cells or cell lines for use in preparing a cDNA
10 library to isolate a cDNA encoding human KDR may be done by first measuring cell-associated KDR activity using any known assay available for such a purpose.

Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library
15 construction techniques can be found for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Complementary DNA libraries may also be obtained from numerous commercial sources, including but not limited to Clontech Laboratories, Inc. and Stratagene.

It is also readily apparent to those skilled in the art that DNA encoding human KDR may also be isolated from a suitable
20 genomic DNA library. Construction of genomic DNA libraries can be performed by standard techniques well known in the art. Well known genomic DNA library construction techniques can be found in
25 Sambrook, et al., *supra*.

In order to clone the human KDR gene by one of the preferred methods, the amino acid sequence or DNA sequence of human KDR or a homologous protein may be necessary. To accomplish this, the KDR protein or a homologous protein may be purified and
30 partial amino acid sequence determined by automated sequenators. It is not necessary to determine the entire amino acid sequence, but the linear sequence of two regions of 6 to 8 amino acids can be determined for the PCR amplification of a partial human KDR DNA fragment. Once suitable amino acid sequences have been identified, the DNA
35 sequences capable of encoding them are synthesized. Because the genetic code is degenerate, more than one codon may be used to encode a

particular amino acid, and therefore, the amino acid sequence can be encoded by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to the human KDR sequence but others in the set will be capable of hybridizing to human KDR DNA even in the presence of DNA oligonucleotides with mismatches. The mismatched DNA oligonucleotides may still sufficiently hybridize to the human KDR DNA to permit identification and isolation of human KDR encoding DNA. Alternatively, the nucleotide sequence of a region of an expressed sequence may be identified by searching one or more available genomic databases. Gene-specific primers may be used to perform PCR amplification of a cDNA of interest from either a cDNA library or a population of cDNAs. As noted above, the appropriate nucleotide sequence for use in a PCR-based method may be obtained from SEQ ID NO: 1, either for the purpose of isolating overlapping 5' and 3' RACE products for generation of a full-length sequence coding for human KDR, or to isolate a portion of the nucleotide sequence coding for human KDR for use as a probe to screen one or more cDNA- or genomic-based libraries to isolate a full-length sequence encoding human KDR or human KDR-like proteins.

In an exemplified method, the human KDR full-length cDNA of the present invention was generated by screening a human umbilical vein endothelial cell (HUVEC) lambda phage cDNA library with a KDR-specific 576 base pair DNA probe prepared by using primers KDR-A: 5'-GGAATTCCATCCAAGCGGCAAATGTGTC-3' (SEQ ID NO:3) and KDR-B: 5'-GGAATTCCGAGTCTTCTACAAGGGTCTC-3' (SEQ ID NO:4). Lambda phage clones containing unique inserts were isolated through three rounds of replating and then characterized. The 3' 110 base pairs not represented in any of the isolated clones were cloned by PCR from the same library as above using the primers KDR-C: 5'-TTATGACAACACAGCAGG-3' (SEQ ID NO:5) and KDR-D: 5'-TTGGATCCTCGAGTTGGGGTGTGGATGC-3' (SEQ ID NO:6). Overlapping clones were used to generate a full-length KDR gene into plasmid vector pGEM7Z. The gene contained an XhoI site at the 5' end which was changed to a BamHI site by first cutting with XhoI, then forming a blunt end with DNA polymerase and ligating an oligonucleotide BamHI linker and finally cloned as a BamHI/BamHI

fragment back into pGEM7Z. The gene was sequenced on an ABI Prism automatic sequencer model number 377. In addition, the cytoplasmic domain of KDR which contains tyrosine kinase activity was cloned separately as a GST gene fusion into a baculovirus expression vector to
5 characterize tyrosine kinase activity.

A variety of mammalian expression vectors may be used to express recombinant human KDR in mammalian cells. Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned DNA and the translation of their mRNAs in an
10 appropriate host. Such vectors can be used to express eukaryotic DNA in a variety of hosts such as bacteria, blue green algae, plant cells, insect cells and animal cells. Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells. An appropriately constructed expression vector should
15 contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one
20 which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses.

Commercially available mammalian expression vectors which may be suitable for recombinant human KDR expression, include
25 but are not limited to, pcDNA3.1 (Invitrogen), pLITMUS28, pLITMUS29, pLITMUS38 and pLITMUS39 (New England Biolabs), pcDNA1, pcDNA1amp (Invitrogen), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224),
30 pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and λ ZD35 (ATCC 37565).

A variety of bacterial expression vectors may be used to express recombinant human KDR in bacterial cells. Commercially available bacterial expression vectors which may be suitable for
35 recombinant human KDR expression include, but are not limited to

pCR2.1 (Invitrogen), pET11a (Novagen), lambda gt11 (Invitrogen), and pKK223-3 (Pharmacia).

5 A variety of fungal cell expression vectors may be used to express recombinant human KDR in fungal cells. Commercially available fungal cell expression vectors which may be suitable for recombinant human KDR expression include but are not limited to pYES2 (Invitrogen) and *Pichia* expression vector (Invitrogen).

10 A variety of insect cell expression vectors may be used to express recombinant receptor in insect cells. Commercially available insect cell expression vectors which may be suitable for recombinant expression of human KDR include but are not limited to pBlueBacIII and pBlueBacHis2 (Invitrogen), and pAcG2T (Pharmingen).

15 An expression vector containing DNA encoding a human KDR-like protein may be used for expression of human KDR in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila*- and silkworm-derived cell
20 lines. Cell lines derived from mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M(TK-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), Saos-2 (ATCC HTB-85), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171) and CPAE (ATCC CCL 209).

30 The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, protoplast fusion, and electroporation. The expression vector-containing cells are individually analyzed to determine whether they produce human KDR protein. Identification of human KDR expressing cells may be done by several means, including but not limited to immunological reactivity with anti-human KDR
35 antibodies, labeled ligand binding and the presence of host cell-associated human KDR activity.

The cloned human KDR cDNA obtained through the methods described above may be recombinantly expressed by molecular cloning into an expression vector (such as pcDNA3.1, pCR2.1, pBlueBacHis2 and pLITMUS28) containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant human KDR. Techniques for such manipulations can be found described in Sambrook, et al., *supra*, are discussed at length in the Example section and are well known and easily available to the artisan of ordinary skill in the art.

Expression of human KDR DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

To determine the human KDR cDNA sequence(s) that yields optimal levels of human KDR, cDNA molecules including but not limited to the following can be constructed: a cDNA fragment containing the full-length open reading frame for human KDR as well as various constructs containing portions of the cDNA encoding only specific domains of the protein or rearranged domains of the protein. All constructs can be designed to contain none, all or portions of the 5' and/or 3' untranslated region of a human KDR cDNA. The expression levels and activity of human KDR can be determined following the introduction, both singly and in combination, of these constructs into appropriate host cells. Following determination of the human KDR cDNA cassette yielding optimal expression in transient assays, this KDR cDNA construct is transferred to a variety of expression vectors (including recombinant viruses), including but not limited to those for mammalian cells, plant cells, insect cells, oocytes, bacteria, and yeast cells.

Levels of human KDR in host cells is quantified by a variety of techniques including, but not limited to, immunoaffinity and/or ligand affinity techniques. KDR-specific affinity beads or KDR-specific

antibodies are used to isolate ³⁵S-methionine labeled or unlabelled KDR. Labeled KDR protein is analyzed by SDS-PAGE. Unlabelled KDR protein is detected by Western blotting, ELISA or RIA assays employing either KDR protein specific antibodies and/or antiphosphotyrosine antibodies.

5 Following expression of KDR in a host cell, KDR protein may be recovered to provide KDR protein in active form. Several KDR protein purification procedures are available and suitable for use. Recombinant KDR protein may be purified from cell lysates and extracts, or from conditioned culture medium, by various combinations
10 of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography.

 In addition, recombinant KDR protein can be separated
15 from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full-length KDR protein, or polypeptide fragments of KDR protein. Additionally, polyclonal or monoclonal antibodies may be raised against a synthetic peptide (usually from about 9 to about 25 amino acids in length) from a
20 portion of the protein as disclosed in SEQ ID NO:2. Monospecific antibodies to human KDR are purified from mammalian antisera containing antibodies reactive against human KDR or are prepared as monoclonal antibodies reactive with human KDR using the technique of Kohler and Milstein (1975, *Nature* 256: 495-497). Monospecific antibody
25 as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for human KDR. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated with human KDR, as described above. Human KDR-specific
30 antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with an appropriate concentration of human KDR protein or a synthetic peptide generated from a portion of human KDR with or without an immune adjuvant.

 Preimmune serum is collected prior to the first
35 immunization. Each animal receives between about 0.1 µg and about 1000 µg of human KDR protein associated with an acceptable immune

adjuvant. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The initial immunization consists of human KDR protein or peptide fragment
5 thereof in, preferably, Freund's complete adjuvant at multiple sites either subcutaneously (SC), intraperitoneally (IP) or both. Each animal is bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. Those animals receiving booster injections
10 are generally given an equal amount of human KDR in Freund's incomplete adjuvant by the same route. Booster injections are given at about three week intervals until maximal titers are obtained. At about 7 days after each booster immunization or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots
15 are stored at about -20°C.

Monoclonal antibodies (mAb) reactive with human KDR are prepared by immunizing inbred mice, preferably Balb/c, with human KDR protein. The mice are immunized by the IP or SC route with about 1 µg to about 100 µg, preferably about 10 µg, of human KDR
20 protein in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant, as discussed above. Freund's complete adjuvant is preferred. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of about 1 to about 100 µg of
25 human KDR in a buffer solution such as phosphate buffered saline by the intravenous (IV) route. Lymphocytes, from antibody positive mice, preferably splenic lymphocytes, are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an
30 appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable hybridomas. Fusion partners may include, but are not limited to: mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0, with Sp 2/0 being preferred. The antibody producing cells and myeloma cells are fused in polyethylene glycol,
35 about 1000 mol. wt., at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine,

thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells on about days 14, 18, and 21 and are screened for antibody production by an immunoassay such as solid
5 phase immunoradioassay (SPIRA) using human KDR as the antigen. The culture fluids are also tested in the Ouchterlony precipitation assay to determine the isotype of the mAb. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, 1973, *Soft Agar Techniques*, in *Tissue Culture Methods and Applications*, Kruse and Paterson, Eds., Academic Press.
10

Monoclonal antibodies are produced *in vivo* by injection of pristine primed Balb/c mice, approximately 0.5 ml per mouse, with about 2×10^6 to about 6×10^6 hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer
15 and the monoclonal antibodies are purified by techniques known in the art.

In vitro production of anti-human KDR mAb is carried out by growing the hybridoma in DMEM containing about 2% fetal calf serum to obtain sufficient quantities of the specific mAb. The mAb are
20 purified by techniques known in the art.

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and
25 radioimmunoassay (RIA) techniques. Similar assays are used to detect the presence of human KDR in body fluids or tissue and cell extracts.

It is readily apparent to those skilled in the art that the above described methods for producing monospecific antibodies may be utilized to produce antibodies specific for human KDR peptide
30 fragments, or full-length human KDR.

Human KDR antibody affinity columns are made, for example, by adding the antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead support.
35 The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M

ethanolamine HCl (pH 8). The column is washed with water followed by 0.23 M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in phosphate buffered saline (pH 7.3) and the cell culture supernatants or cell extracts
5 containing full-length human KDR or human KDR protein fragments are slowly passed through the column. The column is then washed with phosphate buffered saline until the optical density (A₂₈₀) falls to background, then the protein is eluted with 0.23 M glycine-HCl (pH 2.6). The purified human KDR protein is then dialyzed against phosphate
10 buffered saline.

The human KDR protein of the present invention is suitable for use in an assay procedure for the identification of compounds which modulate KDR activity. A KDR-containing fusion construct, such as a GST-KDR fusion as discussed within this specification, is useful to
15 measure KDR activity. Kinase activity is, for example, measured by incorporation of radiolabeled phosphate into polyglutamic acid, tyrosine, 4:1 (pEY) substrate. The phosphorylated pEY product is trapped onto a filter membrane and the incorporation of radiolabeled phosphate quantified by scintillation counting. Soluble recombinant GST-kinase
20 domain fusion proteins are expressed in Sf21 insect cells (Invitrogen) using a baculovirus expression vector (pAcG2T, Pharmingen). A lysis buffer is 50 mM Tris, pH 7.4, 0.5 M NaCl, 5 mM DTT, 1 mM EDTA, 0.5% Triton X-100, 10% glycerol, 10 µg/ml of each leupeptin, pepstatin and aprotinin and 1 mM phenylmethylsulfonyl fluoride (all Sigma). A wash
25 buffer is 50 mM Tris, pH 7.4, 0.5 M NaCl, 5 mM DTT, 1 mM EDTA, 0.05% Triton X-100, 10% glycerol, 10 µg/ml of each leupeptin, pepstatin and aprotinin and 1 mM phenylmethylsulfonyl fluoride. A dialysis buffer is 50 mM Tris, pH 7.4, 0.5 M NaCl, 5 mM DTT, 1 mM EDTA, 0.05% Triton X-100, 50% glycerol, 10 µg/ml of each leupeptin, pepstatin
30 and aprotinin and 1 mM phenylmethylsulfonyl fluoride. A 10X reaction buffer is 200 mM Tris, pH 7.4, 1.0 M NaCl, 50 mM MnCl₂, 10 mM DTT and 5 mg/ml bovine serum albumin (Sigma). An enzyme dilution buffer is 50 mM Tris, pH 7.4, 0.1 M NaCl, 1 mM DTT, 10% glycerol, 100 mg/ml BSA. A 10X substrate solution would be 750 µg/ml poly(glutamic acid,
35 tyrosine; 4:1) (Sigma); stop solution is 30% trichloroacetic acid, 0.2 M sodium pyrophosphate (both Fisher) and wash solution is 15%

trichloroacetic acid, 0.2 M sodium pyrophosphate. The filter plates are Millipore #MAFC NOB, GF/C glass fiber 96 well plates.

First, Sf21 cells are infected with recombinant virus at a multiplicity of infection of 5 virus particles/cell and grown at 27 °C for 48 hours. All subsequent steps are performed at 4 °C. Infected cells are harvested by centrifugation at 1000 X g and lysed at 4 °C for 30 minutes with 1/10 volume of lysis buffer followed by centrifugation at 100,000Xg for 1 hour. The supernatant is then passed over a glutathione-Sepharose column (Pharmacia) equilibrated in lysis buffer and washed with 5 volumes of the same buffer followed by 5 volumes of wash buffer. Recombinant GST-KDR protein is eluted with wash buffer/10 mM reduced glutathione (Sigma) and dialyzed against dialysis buffer.

The KDR assay comprises the following steps:

1. Add 5 µl of inhibitor or control to the assay in 50% DMSO;
2. Add 35 µl of reaction mix containing 5 µl of 10 X reaction buffer, 5 µl 25 mM ATP/10 µCi [³³P]ATP (Amersham), and 5 µl 10 X substrate;
3. Start the reaction by the addition of 10 µl of KDR (25 nM) in enzyme dilution buffer;
4. Mix and incubate at room temperature (~22 °C) for 15 minutes;
5. Stop by the addition of 50 µl stop solution;
6. Incubate for 15 minutes at 4 °C;
7. Transfer a 90 µl aliquot to filter plate;
8. Aspirate and wash 3 times with 100 µl of wash solution;
9. Add 30 µl of scintillation cocktail, seal plate and count in a Wallac Microbeta scintillation counter.

Modulating KDR includes the inhibition or activation of the kinase which affects the mitogenic function of VEGF. Compounds which modulate KDR include agonists and antagonists.

Therefore, the human KDR protein of the present invention may be obtained from both native and recombinant sources (as a full-length protein, biologically active protein fragment, or fusion construction) for use in an assay procedure to identify human KDR modulators. In general, an assay procedure to identify human KDR modulators will contain the intracellular domain of human KDR, and a

test compound or sample which contains a putative KDR kinase agonist or antagonist. The test compounds or samples may be tested directly on, for example, purified KDR, KDR kinase or a GST-KDR kinase fusion, subcellular fractions of KDR-producing cells whether native or recombinant, whole cells expressing human KDR whether native or recombinant, intracellular KDR protein fragments and respective deletion fragments, and/or extracellular intracellular KDR protein fragments and respective deletion fragments. The test compound or sample may be added to KDR in the presence or absence of a known human KDR substrate. The modulating activity of the test compound or sample may be determined by, for example, analyzing the ability of the test compound or sample to bind to the KDR intracellular domain, activate the protein, inhibit the protein, inhibit or enhance the binding of other compounds to human KDR, modifying VEGF receptor regulation, or modifying kinase activity.

Therefore, the present invention also relates to subcellular membrane fractions of the recombinant host cells (both prokaryotic and eukaryotic as well as both stably and transiently transformed cells) comprising the nucleic acids of the present invention. These subcellular membrane fractions will comprise human KDR at levels substantially above wild-type levels and hence will be useful in various assays described throughout this specification.

The identification of modulators of human KDR will be useful in treating various disease states. For example, vascular growth in or near the retina leads to visual degeneration culminating in blindness. VEGF accounts for most of the angiogenic activity produced in or near the retina in diabetic retinopathy. Ocular VEGF mRNA and protein are elevated by conditions such as retinal vein occlusion in primates and decreased pO_2 levels in mice that lead to neovascularization. Expression of VEGF is also significantly increased in hypoxic regions of animal and human tumors adjacent to areas of necrosis. VEGF contributes to tumor growth *in vivo* by promoting angiogenesis through its paracrine vascular endothelial cell chemotactic and mitogenic activities. Inhibition of KDR is implicated in pathological neoangiogenesis, and compounds which inhibit the mitogenic activity of VEGF via inhibition of KDR will be useful in the

treatment of diseases in which neoangiogenesis is part of the overall pathology, such as diabetic retinal vascularization, various forms of cancer and inflammation which demonstrate high levels of gene and protein expression. Examples of such cancers include cancers of the
5 brain, breast, genitourinary tract, lymphatic system, stomach, intestines including colon, pancreas, prostate, larynx and lung. These include histiocytic lymphoma, lung adenocarcinoma, glioblastoma and small cell lung cancers. Examples of inflammation include
10 rheumatoid arthritis, psoriasis, contact dermatitis and hypersensitivity reactions.

The present invention also relates to gene transfer of a DNA vector and concomitant *in vivo* expression of an extracellular, soluble form of human KDR, preferably comprising from about amino acid 1 to from about amino acid 644 (to encompass the initial six IG-like
15 extracellular domains) to about amino acid 763 (to encompass all seven IG-like extracellular domains) of human KDR as set forth in SEQ ID NO:2. Such a gene therapy vehicle will express this soluble form of human KDR, which binds VEGF or a VEGF homologue in and around the localized site of the disorder. The formation of a sKDR/VEGF
20 complex will inhibit binding of VEGF to the KDR and FLT-1 tyrosine kinase receptors spanning the vascular endothelial cell membrane, thus preventing initiation of the signal transduction stimulating angiogenesis. In addition, expression of sKDR may also impart a therapeutic effect by binding to membrane associated VEGF receptors.
25 VEGF receptors are thought to be dimerized by binding dimeric VEGF ligand which in turn allows the receptor intracellular tyrosine kinase domains to transphosphorylate each other generating phosphorylated tyrosine residues that facilitate the subsequent binding and activation of downstream signal transduction proteins. Soluble KDR will be able to
30 form heterodimers with full-length VEGF receptors that, because the sKDR forms are devoid of an intracellular tyrosine kinase region, prevent receptor tyrosine kinase domain transphosphorylation, the initiation of signal transduction and thus VEGF-induced mitogenesis and angiogenesis in a dominant negative manner. The skilled artisan
35 will be able to generate various gene therapy constructs which express various regions of the extracellular domain of KDR for administration to

the patient. While the patient may be any mammalian host, the preferable treatment is directed toward humans. Any such construct will express a KDR fragment which effectively inhibits mitogenic activity associated with VEGF/KDR associations on human endothelial cells. It is preferred in the present invention that this region comprise an isolated nucleic acid molecule which encodes from about amino acid 1 to about amino acid 644 and/or from about amino acid 1 to about amino acid 763 as set forth in SEQ ID NO:2.

Another preferred embodiment of the present invention is a nucleic acid molecule which encodes an extracellular-transmembrane KDR protein fragment which is also useful in gene therapy applications as described in the previous paragraph. It is preferred that any such DNA molecule comprise a DNA sequence from which encodes from about amino acid 1 to about amino acid about the initial 785 - 795 amino acids of KDR as set forth in SEQ ID NO:2, and especially preferred is an isolated nucleic acid molecule construction which encodes the amino terminal portion of KDR with a truncation at about amino acid 791 as set forth in SEQ ID NO:2.

One preferred gene therapy application for the human KDR gene and protein of the present invention relates to promoting inhibition of solid tumor angiogenesis and metastasis by utilizing the disclosed gene therapy methodology. A second preferred gene therapy application for the human KDR gene and protein of the present invention relates to promoting inhibition of diabetic retinopathy, as described elsewhere within this specification. The transferred sKDR nucleic acid is expressed within the region of interest subsequent to gene transfer such that expressed sKDR binds to VEGF to prevent binding of VEGF to the KDR and FLT-1 tyrosine kinase receptors, antagonizing transduction of the normal intracellular signals associated with vascular endothelial cell-induced tumor angiogenesis and diabetic retinopathy.

The present invention is also directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding a human KDR protein. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding

human KDR, or the function of human KDR. Compounds that modulate the expression of DNA or RNA encoding human KDR or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a
5 change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample. Kits containing human KDR, antibodies to human KDR, or modified human KDR may be prepared by known methods for such uses.

10 The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of human KDR. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of human KDR.
15 Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant KDR or anti-KDR antibodies suitable for detecting human KDR. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the
20 like.

Pharmaceutically useful compositions comprising modulators of human KDR may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be
25 found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein, DNA, RNA, modified human KDR, or either KDR agonists or antagonists including tyrosine kinase activators or inhibitors.

30 Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose disorders. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular.

5 The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described
10 in a variety of texts, such as Remington's Pharmaceutical Sciences.

Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents may be desirable.

15 The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient can be administered
20 in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by
25 injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compounds of the present invention may
30 be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well
35 known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will,

of course, be continuous rather than intermittent throughout the dosage regimen.

For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular function of the patient; and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

The following examples are provided to illustrate the present invention without, however, limiting the same hereto.

EXAMPLE 1

Isolation of a cDNA Encoding Human KDR

Materials - A human umbilical vein endothelial cell lambda phage cDNA library was purchased from Clontech (Cat. # HL1070b). DNA modification and restriction enzymes were purchased from Promega. Plasmid pGEM7Z was purchased from Promega (Cat. # P2251). Taq polymerase was from Perkin Elmer Cetus (part number N801-0055). BamHI linkers were purchased from New England Biolabs (Cat. # 1071). [α -³²P] dATP was purchased from Amersham (Cat. # PB 10204). Rediprime was also purchased from Amersham (Cat. # RPN 1633). The baculovirus expression vector pAcG2T was purchased from Pharmingen (Cat. # 21414P).

The PCR primers used are as follows:

KDR-A 5'-GGAATTCCATCCAAGCGGCAAATGTGTC-3' (SEQ ID NO:3);

KDR-B 5'-GGAATTCCGAGTCTTCTACAAGGGTCTC-3' (SEQ ID NO:4)

5 KDR-C 5'-TTATGACAACACAGCAGG-3' (SEQ ID NO:5); and,

KDR-D 5'-TTGGATCCTCGAGTTGGGGTGTGGATGC-3' (SEQ ID NO:6).

Methods: Gene Cloning - The KDR cDNA was isolated by probing a human umbilical vein endothelial cell lambda phage cDNA library from Clontech with a KDR-specific 576 base pair DNA probe. The probe was prepared by PCR using primers KDR-A/KDR-B and Taq polymerase, then labeled to a specific activity of 1×10^7 cpm/ng by random priming. Phage were plated at about 50,000 plaques/plate and hybridization was done by standard protocols. A total of 1×10^6 phage were screened. Lambda phage clones containing unique inserts were isolated through three rounds of replating and then characterized. The 3' 110 base pairs not represented in any of the isolated clones were cloned by PCR from the same library as above using the primers KDR-C and KDR-D. Overlapping clones were used to generate a full-length KDR gene by restriction enzyme digestion, isolation of the individual gene fragments and ligation (restriction enzymes and ligase were from Promega) into pGEM7Z. The gene contained an XhoI site at the 5' end which was changed to a BamHI site by first cutting with XhoI, then forming a blunt end with DNA polymerase and ligating an oligonucleotide BamHI linker and finally cloned as a BamHI/BamHI fragment back into pGEM7Z. The gene was sequenced on an ABI Prism automatic sequencer model number 377. The cDNA sequence of human KDR is shown in Figure 1A and 1B. The deduced amino acid sequence of human KDR is shown in Figure 2.

30

EXAMPLE 2

Construction of GST/KDR-1

The cytoplasmic domain of KDR which contains tyrosine kinase activity was cloned separately as a glutathione S-transferase (GST) gene fusion into a baculovirus expression vector to characterize

35

tyrosine kinase activity. To construct this GST fusion, a Kpn I cloning site was introduced into the KDR gene by changing the codons encoding residues Gly 800 (GGG to GGC) and Leu 802 (TTG to CTG) and the existing BamHI site was removed by changing the codon encoding Asp 807 (GAT to GAC); these changes are silent and do not change the amino acid sequence of the receptor. A new BamHI site was introduced to form an in frame fusion with the carboxyl terminus of GST and KDR at Ala 792. The GST and KDR BamHI-digested fragments were ligated to generate the in frame GST/KDR fusion. Active GST-KDR tyrosine kinase protein is produced in insect cells.

EXAMPLE 3

Construction Of KDR Core Kinase Domain

The kinase domain of KDR was cloned using the preexisting BamHI site at the 5' end of the kinase domain and introducing a stop codon followed by a SalI site at the 3' end of the kinase domain (Tyr 1175 TAC changed to TAA). KDR DNA was used as a template in a PCR reaction with primers KDR-E (5'-GGATCCAGATGAACTCCCATTTG-3' [SEQ ID NO:7]) and KDR-F (5'-GTCGACTTAGTCTTTGCCATCCTGCTGAGC-3' [SEQ ID NO:8]). The resulting KDR core kinase BamHI/Sal I fragment was cloned into pBlueBacHis2B, this creates an inframe fusion of the methionine initiator codon and the poly histidine sequence of the vector with the KDR kinase domain. This vector, pBBH-KDR-1, also provides an enterokinase recognition site to remove the His tag polypeptide by proteolysis. The KDR core kinase protein was expressed in insect cells and purified on a nickel chelating column. The purified KDR core kinase was active in the kinase assay described herein.

EXAMPLE 4

Molecular Modeling of Human KDR

The cytoplasmic domain of the VEGF receptor was aligned by hand to the sequence of FGFR1 as taken from the published crystal structure (Mohammadi, M., Schlessinger, J. and Hubbard, S.R., 1996,

Cell 86: 577). The sequences are ~60% identical in this alignment. An
homology model of KDR kinase was then built in Quanta (version 4.1p)
by copying the coordinates from the FGFR1/AMP-PCP crystal structure.
The kinase insert region (residues 933-1006 in KDR) was not included in
5 the model since there was no unique conformation for this region in the
crystal structure. The homology model was then minimized using
CHARMM within Quanta constraining the protein backbone and
allowing the side chains to move freely.

The change of amino acid residue 848 from the published
10 Glu to Val in SEQ ID NO:2 is found in the glycine-rich flap, which forms
part of the ATP binding pocket. The highly conserved Val is found to
form hydrophobic contacts to ATP in other kinases, and appears to be
positioned to form these same contacts in KDR. A charged Glu in this
position is not likely to make proper contact with ATP. This is shown by
15 computer modeling in Figure 3A and Figure 3B. Figure 3A shows the
ATP binding domain from the KDR V848E mutant homology model with
bound AMP-PCP. The side chain of E848 is in contact the adenine from
AMP-PCP. The gamma phosphate of AMP-PCP is not visible. The
protein carbon alpha trace is shown in pipes, the AMP-PCP in sticks
20 and the E848 side chain in space filling. The N-terminal lobe is colored
blue (or alternatively labeled with light circles) with the exception of the
glycine rich flap which is colored green (or alternatively labeled as a
lined region). The C-terminal lobe is colored red (or alternatively labeled
with dark circles). Figure 3B shows ATP binding domain from the KDR
25 homology model with bound AMP-PCP. The side chain of V848 forms
hydrophobic contacts with the adenine from AMP-PCP. The gamma
phosphate of AMP-PCP is not visible. The protein carbon alpha trace is
shown in pipes, the AMP-PCP in sticks and the V848 side chain in space
filling. The N-terminal lobe is colored blue (or alternatively labeled with
30 light circles) with the exception of the glycine rich flap which is colored
green (or alternatively labeled as a lined region). The C-terminal lobe is
colored red (or alternatively labeled with dark circles).

EXAMPLE 5

Tyrosine Phosphorylation of KDR_{cyt} Mutants

Purified KDR_{cyt}E848 and KDR_{cyt}V848 were incubated with
5 at concentrations of 12 ng or 120 ng, respectively, or without 1 mM ATP
at 37 °C for 10 min. The reaction was stopped by the addition of an equal
volume of 2X SDS-PAGE sample buffer and boiled for 5 min. Reaction
products were separated by 7.5%/SDS-PAGE and analyzed by Western
10 blot probed with the antiphosphotyrosine antibody PY20 (Transduction
Laboratories; Figure 4A), or an anti-KDR antibody (Santa Cruz
Biotechnology; Figure 4B) visualized using the ECL detection kit and
quantified by scanning with a densitometer (Molecular Dynamics).
Figure 4A shows that purified GST-KDR_{cyt}E848 was unable to
autophosphorylate in the presence of 1-mM ATP wherein 12 ng of GST-
15 KDR_{cyt}V848 in the presence of 1 mM ATP resulted in
autophosphorylation. Figure 4B shows a signal against anti-KDR
antibody for 120 ng GST-KDR_{cyt}E848 and 12 ng of GST-KDR_{cyt}V848.

SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION:
- (i) APPLICANTS: Merck & Co., Inc.
- 10 (ii) TITLE OF INVENTION: HUMAN RECEPTOR TYROSINE KINASE, KDR
- (iii) NUMBER OF SEQUENCES: 8
- (iv) CORRESPONDENCE ADDRESS:
- 15 (A) ADDRESSEE: Merck & Co., Inc.
- (B) STREET: P.O. Box 2000
- (C) CITY: Rahway
- (D) STATE: NJ
- (E) COUNTRY: US
- 20 (F) ZIP: 07065-0907
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- 25 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
- 30 (B) FILING DATE:
- (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Hand, J. Mark
- 35 (B) REGISTRATION NUMBER: 36,545
- (C) REFERENCE/DOCKET NUMBER: 19963PV
- (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: 732/594-3905
- 40 (B) TELEFAX: 732/594-4720
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
- 45 (A) LENGTH: 4071 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- 50 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

	ATGGAGAGCA AGGTGCTGCT GGCCGTCGCC CTGTGGCTCT GCGTGGAGAC CCGGGCCGCC	60
5	TCTGTGGGTT TGCCTAGTGT TTCTCTTGAT CTGCCCAGGC TCAGCATACA AAAAGACATA	120
	CTTACAATTA AGGCTAATAC AACTCTTCAA ATTACTTGCA GGGGACAGAG GGA CTGAGC	180
10	TGGCTTTGGC CCAATAATCA GAGTGGCAGT GAGCAAAGGG TGGAGGTGAC TGAGTGCAGC	240
	GATGGCCTCT TCTGTAAGAC ACTCACAATT CCAAAAGTGA TCGGAAATGA CACTGGAGCC	300
	TACAAGTGCT TCTACCGGA AACTGACTTG GCCTCGGTCA TTTATGTCTA TGTTCAGAT	360
15	TACAGATCTC CATTTATTGC TTCTGTTAGT GACCAACATG GAGTCGTGTA CATTACTGAG	420
	AACAAAAACA AAAGTGTGGT GATTCCATGT CTCGGGTCCA TTCAAATCT CAACGTGTCA	480
20	CTTTGTGCAA GATACCCAGA AAAGAGATTT GTTCCTGATG GTAACAGAAT TTCCTGGGAC	540
	AGCAAGAAGG GCTTTACTAT TCCCAGCTAC ATGATCAGCT ATGCTGGCAT GGTCTTCTGT	600
	GAAGCAAAAA TTAATGATGA AAGTTACCAG TCTATTATGT ACATAGTTGT CGTTGTAGGG	660
25	TATAGGATTT ATGATGTGGT TCTGAGTCCG TCTCATGGAA TTGAACTATC TGTGAGAGAA	720
	AAGCTTGTCT TAAATTGTAC AGCAAGAAGT GAAGTAAATG TGGGGATTGA CTTCAACTGG	780
30	GAATACCCTT CTTCGAAGCA TCAGCATAAG AAAGTGTGTA ACCGAGACCT AAAAAGCCAG	840
	TCTGGGAGTG AGATGAAGAA ATTTTGTAGC ACCTTAACTA TAGATGGTGT AAGCCGAGT	900
	GACCAAGGAT TGTACACCTG TGCAGCATCC AGTGGGCTGA TGACCAAGAA GAACAGCACA	960
35	TTTGTGAGGG TCCATGAAAA ACCTTTTGTG GCTTTTGGAA GTGGCATGGA ATCTCTGGTG	1020
	GAAGCCACGG TGGGGGAGCG TGTGAGAATC CCTGCGAAGT ACCTTGTTTA CCCACCCCA	1080
40	GAAATAAAAT GGTATAAAAA TGGAATACCC CTTGAGTCCA ATCACACAAT TAAAGCGGGG	1140
	CATGTACTGA CGATTATGGA AGTGAGTGAA AGAGACACAG GAAATTACAC TGTATCCTT	1200
	ACCAATCCCA TTTCAAAGGA GAAGCAGAGC CATGTGGTCT CTCTGGTTGT GTATGTCCCA	1260
45	CCCCAGATTG GTGAGAAATC TCTAATCTCT CCTGTGGATT CCTACCAGTA CGGCACCACT	1320
	CAAACGCTGA CATGTACGGT CTATGCCATT CCTCCCCGC ATCACATCCA CTGGTATTGG	1380
50	CAGTTGGAGG AAGAGTGC GC CAACGAGCCC AGCCAAGCTG TCTCAGTGAC AAACCCATAC	1440
	CCTTGTGAAG AATGGAGAAG TGTGGAGGAC TTCCAGGGAG GAAATAAAAT TGAAGTTAAT	1500
	AAAAATCAAT TTGCTCTAAT TGAAGGAAAA AACAAAAGT TAAGTACCCT TGTATCCAA	1560
55	GCGGCAAATG TGTGAGCTTT GTACAAATGT GAAGCGGTCA ACAAAGTCGG GAGAGGAGAG	1620
	AGGGTGATCT CCTTCCACGT GACCAGGGGT CCTGAAATTA CTTTGCAACC TGACATGCAG	1680

	CCCACTGAGC	AGGAGAGCGT	GTCTTTGTGG	TGCACTGCAG	ACAGATCTAC	GTTTGAGAAC	1740
	CTCACATGGT	ACAAGCTTGG	CCCACAGCCT	CTGCCAATCC	ATGTGGGAGA	GTTGCCCCACA	1800
5	CCTGTTTGCA	AGAACTTGGA	TACTCTTTGG	AAATTGAATG	CCACCATGTT	CTCTAATAGC	1860
	ACAAATGACA	TTTTGATCAT	GGAGCTTAAG	AATGCATCCT	TGCAGGACCA	AGGAGACTAT	1920
10	GTCTGCCTTG	CTCAAGACAG	GAAGACCAAG	AAAAGACATT	GCGTGGTCAG	GCAGCTCACA	1980
	GTCCTAGAGC	GTGTGGCACC	CACGATCACA	GGAAACCTGG	AGAATCAGAC	GACAAGTATT	2040
	GGGGAAAGCA	TCGAAGTCTC	ATGCACGGCA	TCTGGGAATC	CCCCTCCACA	GATCATGTGG	2100
15	TTTAAAGATA	ATGAGACCCT	TGTAGAAGAC	TCAGGCATTG	TATTGAAGGA	TGGGAACCGG	2160
	AACCTCACTA	TCCGCAGAGT	GAGGAAGGAG	GACGAAGGCC	TCTACACCTG	CCAGGCATGC	2220
20	AGTGTTCCTG	GCTGTGCAAA	AGTGGAGGCA	TTTTTCATAA	TAGAAGGTGC	CCAGGAAAAG	2280
	ACGAACTTGG	AAATCATTAT	TCTAGTAGGC	ACGGCGGTGA	TTGCCATGTT	CTTCTGGCTA	2340
	CTTCTTGTC	TCATCCTACG	GACCGTTAAG	CGGGCCAATG	GAGGGGAACT	GAAGACAGGG	2400
25	TACCTGTCCA	TCGTCATGGA	TCCAGATGAA	CTCCCATTGG	ATGAACATTG	TGAACGACTG	2460
	CCTTATGATG	CCAGCAAATG	GGAATTCCCC	AGAGACCGGC	TGAAGCTAGG	TAAGCCTCTT	2520
30	GGCCGTGGTG	CCTTTGGCCA	AGTGATTGAA	GCAGATGCCT	TTGGAATTGA	CAAGACAGCA	2580
	ACTTGCAGGA	CAGTAGCAGT	CAAAATGTTG	AAAGAAGGAG	CAACACACAG	TGAGCATCGA	2640
	GCTCTCATGT	CTGAACTCAA	GATCCTCATT	CATATTGGTC	ACCATCTCAA	TGTGGTCAAC	2700
35	CTTCTAGGTG	CCTGTACCAA	GCCAGGAGGG	CCACTCATGG	TGATTGTGGA	ATTCTGCAAA	2760
	TTTGGAACC	TGTCCACTTA	CCTGAGGAGC	AAGAGAAATG	AATTTGTCCC	CTACAAGACC	2820
40	AAAGGGGCAC	GATTCCGTCA	AGGGAAAGAC	TACGTTGGAG	CAATCCCTGT	GGATCTGAAA	2880
	CGGCGCTTGG	ACAGCATCAC	CAGTAGCCAG	AGCTCAGCCA	GCTCTGGATT	TGTGGAGGAG	2940
	AAGTCCCTCA	GTGATGTAGA	AGAAGAGGAA	GCTCCTGAAG	ATCTGTATAA	GGACTTCCTG	3000
45	ACCTTGAGC	ATCTCATCTG	TTACAGCTTC	CAAGTGGCTA	AGGGCATGGA	GTTCTTGGCA	3060
	TCGCGAAAGT	GATCCACAG	GGACCTGGCG	GCACGAAATA	TCCTCTTATC	GGAGAAGAAC	3120
50	GTGGTTAAAA	TCTGTGACTT	TGGCTTGGCC	CGGGATATTT	ATAAAGATCC	AGATTATGTC	3180
	AGAAAAGGAG	ATGCTCGCCT	CCCTTTGAAA	TGGATGGCCC	CAGAAACAAT	TTTTGACAGA	3240
	GTGTACACAA	TCCAGAGTGA	CGTCTGGTCT	TTTGGTGT'TT	TGCTGTGGGA	AATATTTTCC	3300
55	TTAGGTGCTT	CTCCATATCC	TGGGGTAAAG	ATTGATGAAG	AATTTTGTAG	GCGATTGAAA	3360
	GAAGGAACTA	GAATGAGGGC	CCCTGATTAT	ACTACACCAG	AAATGTACCA	GACCATGCTG	3420

GACTGCTGGC ACGGGGAGCC CAGTCAGAGA CCCACGTTTT CAGAGTTGGT GGAACATTTG 3480
 GGAAATCTCT TGCAAGCTAA TGCTCAGCAG GATGGCAAAG ACTACATTGT TCTTCCGATA 3540
 5 TCAGAGACTT TGAGCATGGA AGAGGATTCT GGA CTCTCTC TGCCTACCTC ACCTGTTTCC 3600
 TGTATGGAGG AGGAGGAAGT ATGTGACCCC AAATTCCATT ATGACAACAC AGCAGGAATC 3660
 10 AGTCAGTATC TGCAGAACAG TAAGCGAAAG AGCCGGCCTG TGAGTGTAAG AACATTTGAA 3720
 GATATCCCGT TAGAAGAACC AGAAGTAAAA GTAATCCCAG ATGACAACCA GACGGACAGT 3780
 GGTATGGTTC TTGCCTCAGA AGAGCTGAAA ACTTTGGAAG ACAGAACCAA ATTATCTCCA 3840
 15 TCTTTTGGTG GAATGGTGCC CAGCAAAAGC AGGGAGTCTG TGGCATCTGA AGGCTCAAAC 3900
 CAGACAAGCG GCTACCAGTC CGGATATCAC TCCGATGACA CAGACACCAC CGTGTACTCC 3960
 20 AGTGAGGAAG CAGAACTTTT AAAGCTGATA GAGATTGGAG TGCAAACCGG TAGCACAGCC 4020
 CAGATTCTCC AGCCTGACTC GGGGACCACA CTGAGCTCTC CTCCTGTTTA A 4071

(2) INFORMATION FOR SEQ ID NO:2:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1356 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
 Met Glu Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu Cys Val Glu
 1 5 10 15
 35 Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro
 20 25 30
 40 Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
 35 40 45
 Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
 50 55 60
 45 Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
 65 70 75 80
 50 Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
 85 90 95
 Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
 100 105 110
 55 Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
 115 120 125
 Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
 130 135 140

	Thr	Val	Val	Ile	Pro	Cys	Leu	Gly	Ser	Ile	Ser	Asn	Leu	Asn	Val	Ser	145	150	155	160
5	Leu	Cys	Ala	Arg	Tyr	Pro	Glu	Lys	Arg	Phe	Val	Pro	Asp	Gly	Asn	Arg	165	170	175	
	Ile	Ser	Trp	Asp	Ser	Lys	Lys	Gly	Phe	Thr	Ile	Pro	Ser	Tyr	Met	Ile	180	185	190	
10	Ser	Tyr	Ala	Gly	Met	Val	Phe	Cys	Glu	Ala	Lys	Ile	Asn	Asp	Glu	Ser	195	200	205	
15	Tyr	Gln	Ser	Ile	Met	Tyr	Ile	Val	Val	Val	Val	Gly	Tyr	Arg	Ile	Tyr	210	215	220	
	Asp	Val	Val	Leu	Ser	Pro	Ser	His	Gly	Ile	Glu	Leu	Ser	Val	Gly	Glu	225	230	235	240
20	Lys	Leu	Val	Leu	Asn	Cys	Thr	Ala	Arg	Thr	Glu	Leu	Asn	Val	Gly	Ile	245	250	255	
	Asp	Phe	Asn	Trp	Glu	Tyr	Pro	Ser	Ser	Lys	His	Gln	His	Lys	Lys	Leu	260	265	270	
25	Val	Asn	Arg	Asp	Leu	Lys	Thr	Gln	Ser	Gly	Ser	Glu	Met	Lys	Lys	Phe	275	280	285	
30	Leu	Ser	Thr	Leu	Thr	Ile	Asp	Gly	Val	Thr	Arg	Ser	Asp	Gln	Gly	Leu	290	295	300	
	Tyr	Thr	Cys	Ala	Ala	Ser	Ser	Gly	Leu	Met	Thr	Lys	Lys	Asn	Ser	Thr	305	310	315	320
35	Phe	Val	Arg	Val	His	Glu	Lys	Pro	Phe	Val	Ala	Phe	Gly	Ser	Gly	Met	325	330	335	
	Glu	Ser	Leu	Val	Glu	Ala	Thr	Val	Gly	Glu	Arg	Val	Arg	Ile	Pro	Ala	340	345	350	
40	Lys	Tyr	Leu	Gly	Tyr	Pro	Pro	Pro	Glu	Ile	Lys	Trp	Tyr	Lys	Asn	Gly	355	360	365	
45	Ile	Pro	Leu	Glu	Ser	Asn	His	Thr	Ile	Lys	Ala	Gly	His	Val	Leu	Thr	370	375	380	
	Ile	Met	Glu	Val	Ser	Glu	Arg	Asp	Thr	Gly	Asn	Tyr	Thr	Val	Ile	Leu	385	390	395	400
50	Thr	Asn	Pro	Ile	Ser	Lys	Glu	Lys	Gln	Ser	His	Val	Val	Ser	Leu	Val	405	410	415	
	Val	Tyr	Val	Pro	Pro	Gln	Ile	Gly	Glu	Lys	Ser	Leu	Ile	Ser	Pro	Val	420	425	430	
55	Asp	Ser	Tyr	Gln	Tyr	Gly	Thr	Thr	Gln	Thr	Leu	Thr	Cys	Thr	Val	Tyr	435	440	445	

	Ala	Ile	Pro	Pro	Pro	His	His	Ile	His	Trp	Tyr	Trp	Gln	Leu	Glu	Glu	
	450						455					460					
5	Glu	Cys	Ala	Asn	Glu	Pro	Ser	Gln	Ala	Val	Ser	Val	Thr	Asn	Pro	Tyr	
	465					470					475					480	
	Pro	Cys	Glu	Glu	Trp	Arg	Ser	Val	Glu	Asp	Phe	Gln	Gly	Gly	Asn	Lys	
					485					490					495		
10	Ile	Glu	Val	Asn	Lys	Asn	Gln	Phe	Ala	Leu	Ile	Glu	Gly	Lys	Asn	Lys	
				500					505					510			
	Thr	Val	Ser	Thr	Leu	Val	Ile	Gln	Ala	Ala	Asn	Val	Ser	Ala	Leu	Tyr	
15			515					520					525				
	Lys	Cys	Glu	Ala	Val	Asn	Lys	Val	Gly	Arg	Gly	Glu	Arg	Val	Ile	Ser	
		530					535					540					
20	Phe	His	Val	Thr	Arg	Gly	Pro	Glu	Ile	Thr	Leu	Gln	Pro	Asp	Met	Gln	
	545					550					555					560	
	Pro	Thr	Glu	Gln	Glu	Ser	Val	Ser	Leu	Trp	Cys	Thr	Ala	Asp	Arg	Ser	
					565					570					575		
25	Thr	Phe	Glu	Asn	Leu	Thr	Trp	Tyr	Lys	Leu	Gly	Pro	Gln	Pro	Leu	Pro	
				580					585					590			
	Ile	His	Val	Gly	Glu	Leu	Pro	Thr	Pro	Val	Cys	Lys	Asn	Leu	Asp	Thr	
30			595					600					605				
	Leu	Trp	Lys	Leu	Asn	Ala	Thr	Met	Phe	Ser	Asn	Ser	Thr	Asn	Asp	Ile	
		610					615					620					
35	Leu	Ile	Met	Glu	Leu	Lys	Asn	Ala	Ser	Leu	Gln	Asp	Gln	Gly	Asp	Tyr	
	625					630					635					640	
	Val	Cys	Leu	Ala	Gln	Asp	Arg	Lys	Thr	Lys	Lys	Arg	His	Cys	Val	Val	
					645					650					655		
40	Arg	Gln	Leu	Thr	Val	Leu	Glu	Arg	Val	Ala	Pro	Thr	Ile	Thr	Gly	Asn	
				660					665					670			
	Leu	Glu	Asn	Gln	Thr	Thr	Ser	Ile	Gly	Glu	Ser	Ile	Glu	Val	Ser	Cys	
45			675					680					685				
	Thr	Ala	Ser	Gly	Asn	Pro	Pro	Pro	Gln	Ile	Met	Trp	Phe	Lys	Asp	Asn	
		690				695						700					
50	Glu	Thr	Leu	Val	Glu	Asp	Ser	Gly	Ile	Val	Leu	Lys	Asp	Gly	Asn	Arg	
	705					710					715					720	
	Asn	Leu	Thr	Ile	Arg	Arg	Val	Arg	Lys	Glu	Asp	Glu	Gly	Leu	Tyr	Thr	
				725						730					735		
55	Cys	Gln	Ala	Cys	Ser	Val	Leu	Gly	Cys	Ala	Lys	Val	Glu	Ala	Phe	Phe	
				740					745					750			

	Ile	Ile	Glu	Gly	Ala	Gln	Glu	Lys	Thr	Asn	Leu	Glu	Ile	Ile	Ile	Leu	
			755					760					765				
5	Val	Gly	Thr	Ala	Val	Ile	Ala	Met	Phe	Phe	Trp	Leu	Leu	Leu	Val	Ile	
		770					775					780					
	Ile	Leu	Arg	Thr	Val	Lys	Arg	Ala	Asn	Gly	Gly	Glu	Leu	Lys	Thr	Gly	
	785					790					795					800	
10	Tyr	Leu	Ser	Ile	Val	Met	Asp	Pro	Asp	Glu	Leu	Pro	Leu	Asp	Glu	His	
					805					810					815		
	Cys	Glu	Arg	Leu	Pro	Tyr	Asp	Ala	Ser	Lys	Trp	Glu	Phe	Pro	Arg	Asp	
15				820					825					830			
	Arg	Leu	Lys	Leu	Gly	Lys	Pro	Leu	Gly	Arg	Gly	Ala	Phe	Gly	Gln	Val	
			835					840					845				
20	Ile	Glu	Ala	Asp	Ala	Phe	Gly	Ile	Asp	Lys	Thr	Ala	Thr	Cys	Arg	Thr	
	850						855					860					
	Val	Ala	Val	Lys	Met	Leu	Lys	Glu	Gly	Ala	Thr	His	Ser	Glu	His	Arg	
	865					870					875					880	
25	Ala	Leu	Met	Ser	Glu	Leu	Lys	Ile	Leu	Ile	His	Ile	Gly	His	His	Leu	
					885					890					895		
	Asn	Val	Val	Asn	Leu	Leu	Gly	Ala	Cys	Thr	Lys	Pro	Gly	Gly	Pro	Leu	
30				900					905					910			
	Met	Val	Ile	Val	Glu	Phe	Cys	Lys	Phe	Gly	Asn	Leu	Ser	Thr	Tyr	Leu	
		915						920					925				
35	Arg	Ser	Lys	Arg	Asn	Glu	Phe	Val	Pro	Tyr	Lys	Thr	Lys	Gly	Ala	Arg	
	930						935					940					
	Phe	Arg	Gln	Gly	Lys	Asp	Tyr	Val	Gly	Ala	Ile	Pro	Val	Asp	Leu	Lys	
	945					950					955					960	
40	Arg	Arg	Leu	Asp	Ser	Ile	Thr	Ser	Ser	Gln	Ser	Ser	Ala	Ser	Ser	Gly	
					965					970					975		
	Phe	Val	Glu	Glu	Lys	Ser	Leu	Ser	Asp	Val	Glu	Glu	Glu	Glu	Ala	Pro	
45				980					985					990			
	Glu	Asp	Leu	Tyr	Lys	Asp	Phe	Leu	Thr	Leu	Glu	His	Leu	Ile	Cys	Tyr	
		995						1000					1005				
50	Ser	Phe	Gln	Val	Ala	Lys	Gly	Met	Glu	Phe	Leu	Ala	Ser	Arg	Lys	Cys	
	1010						1015					1020					
	Ile	His	Arg	Asp	Leu	Ala	Ala	Arg	Asn	Ile	Leu	Leu	Ser	Glu	Lys	Asn	
	1025					1030					1035					1040	
55	Val	Val	Lys	Ile	Cys	Asp	Phe	Gly	Leu	Ala	Arg	Asp	Ile	Tyr	Lys	Asp	
					1045					1050						1055	

	Pro	Asp	Tyr	Val	Arg	Lys	Gly	Asp	Ala	Arg	Leu	Pro	Leu	Lys	Trp	Met	
				1060					1065					1070			
5	Ala	Pro	Glu	Thr	Ile	Phe	Asp	Arg	Val	Tyr	Thr	Ile	Gln	Ser	Asp	Val	
			1075					1080					1085				
	Trp	Ser	Phe	Gly	Val	Leu	Leu	Trp	Glu	Ile	Phe	Ser	Leu	Gly	Ala	Ser	
		1090					1095					1100					
10	Pro	Tyr	Pro	Gly	Val	Lys	Ile	Asp	Glu	Glu	Phe	Cys	Arg	Arg	Leu	Lys	
	1105					1110					1115					1120	
	Glu	Gly	Thr	Arg	Met	Arg	Ala	Pro	Asp	Tyr	Thr	Thr	Pro	Glu	Met	Tyr	
15					1125					1130					1135		
	Gln	Thr	Met	Leu	Asp	Cys	Trp	His	Gly	Glu	Pro	Ser	Gln	Arg	Pro	Thr	
				1140					1145					1150			
20	Phe	Ser	Glu	Leu	Val	Glu	His	Leu	Gly	Asn	Leu	Leu	Gln	Ala	Asn	Ala	
			1155					1160					1165				
	Gln	Gln	Asp	Gly	Lys	Asp	Tyr	Ile	Val	Leu	Pro	Ile	Ser	Glu	Thr	Leu	
			1170				1175					1180					
25	Ser	Met	Glu	Glu	Asp	Ser	Gly	Leu	Ser	Leu	Pro	Thr	Ser	Pro	Val	Ser	
	1185					1190					1195					1200	
	Cys	Met	Glu	Glu	Glu	Glu	Val	Cys	Asp	Pro	Lys	Phe	His	Tyr	Asp	Asn	
30					1205					1210					1215		
	Thr	Ala	Gly	Ile	Ser	Gln	Tyr	Leu	Gln	Asn	Ser	Lys	Arg	Lys	Ser	Arg	
				1220					1225					1230			
35	Pro	Val	Ser	Val	Lys	Thr	Phe	Glu	Asp	Ile	Pro	Leu	Glu	Glu	Pro	Glu	
			1235					1240					1245				
	Val	Lys	Val	Ile	Pro	Asp	Asp	Asn	Gln	Thr	Asp	Ser	Gly	Met	Val	Leu	
		1250					1255					1260					
40	Ala	Ser	Glu	Glu	Leu	Lys	Thr	Leu	Glu	Asp	Arg	Thr	Lys	Leu	Ser	Pro	
	1265					1270					1275					1280	
	Ser	Phe	Gly	Gly	Met	Val	Pro	Ser	Lys	Ser	Arg	Glu	Ser	Val	Ala	Ser	
45				1285						1290				1295			
	Glu	Gly	Ser	Asn	Gln	Thr	Ser	Gly	Tyr	Gln	Ser	Gly	Tyr	His	Ser	Asp	
				1300					1305					1310			
50	Asp	Thr	Asp	Thr	Thr	Val	Tyr	Ser	Ser	Glu	Glu	Ala	Glu	Leu	Leu	Lys	
		1315						1320					1325				
	Leu	Ile	Glu	Ile	Gly	Val	Gln	Thr	Gly	Ser	Thr	Ala	Gln	Ile	Leu	Gln	
		1330					1335					1340					
55	Pro	Asp	Ser	Gly	Thr	Thr	Leu	Ser	Ser	Pro	Pro	Val					
	1345					1350					1355						

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

10 (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

15 GGAATTCCAT CCAAGCGCA AATGTGTC 28

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

25 (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

30 GGAATTCGA GTCTTCTACA AGGGTCTC 28

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

40 (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

45 TTATGACAAC ACAGCAGG 18

(2) INFORMATION FOR SEQ ID NO:6:

50 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

5 TTGGATCCTC GAGTTGGGGT GTGGATGC 28

(2) INFORMATION FOR SEQ ID NO:7:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

20 GGATCCAGAT GAACTCCCAT TG 22

(2) INFORMATION FOR SEQ ID NO:8:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

35 GTCGACTTAG TCTTTGCCAT CCTGCTGAGC 30

40

WHAT IS CLAIMED:

1. A purified nucleic acid molecule encoding a human KDR protein which consists essentially of the nucleotide sequence

5 ATGGAGAGCAAGGTGCTGCTGGCCGTCGCCCTGTGGCTCTGCGTGGAGACCCGGGCCGCCTCTGTGGGT
TTGCCCTAGTGTTCCTCTTGATCTGCCCAGGCTCAGCATACAAAAAGACATACTTACAATTAAGGCTAAT
ACAACCTCTTCAAATTACTTGCAGGGGACAGAGGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGC
AGTGAGCAAAGGGTGGAGGTGACTGAGTGCAGCGATGGCCTCTTCTGTAAGACACTCACAATTCACAAA
GTGATCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGGAACTGACTTGGCCTCGGTCATTTAT
10 GTCTATGTTCAAGATTACAGATCTCCATTTATTGCTTCTGTAGTGACCAACATGGAGTCGTGTACATT
ACTGAGAACAAAAACAAAACCTGTGGTGATTCCATGTCTCGGGTCCATTTCAAATCTCAACGTGTCACTT
TGTGCAAGATACCCAGAAAAGAGATTTGTTCTGATGGTAACAGAATTTCTTGGGACAGCAAGAAGGGC
TTTACTATTCCCAGCTACATGATCAGCTATGCTGGCATGGTCTTCTGTGAAGCAAAAATTAATGATGAA
AGTTACCAGTCTATTATGTACATAGTTGTCGTTGTAGGGTATAGGATTTATGATGTGGTTCTGAGTCCG
15 TCTCATGGAATTGAACTATCTGTTGGAGAAAAGCTTGTCTTAAATTTGTACAGCAAGAACTGAACTAAAT
GTGGGGATTGACTTCAACTGGGAATACCCCTTCTTGAAGCATCAGCATAAGAACTTGTAAACCGAGAC
CTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAAACCCGG
AGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTTGTC
AGGGTCCATGAAAAACCTTTTGTGCTTTTGAAGTGGCATGGAATCTCTGGTGGGAAGCCACGGTGGGG
20 GAGCGTGTGAGAAATCCCTGCGAAGTACCTTGGTTACCCACCCCCAGAAATAAAATGGTATAAAAATGGA
ATACCCCTTGAGTCCAATCACACAATTAAAGCGGGGCATGTAAGTACGATTATGGAAGTGAGTGAAAGA
GACACAGGAAATTACACTGTCATCCTTACCAATCCCATTTCAAAGGAGAAGCAGAGCCATGTGGTCTCT
CTGGTTGTGTATGTCCACCCAGATTGGTGAGAAATCTCTAATCTCTCTCTGTGGATTCTTACCAGTAC
GGCACCACTCAAACGCTGACATGTACGGTCTATGCCATTCTCTCCCCGCATCACATCCACTGGTATTGG
25 CAGTTGGAGGAAGAGTGCGCCAACGAGCCCAGCCAAGCTGTCTCAGTGACAAACCCATACCTTGTGAA
GAATGGAGAAAGTGTGGAGGACTTCCAGGGAGGAAATAAAATTTGAAGTTAATAAAAATCAATTTGCTCTA
ATTGAAGGAAAAAACAAAACCTGTAAGTACCCTTGTATCCAAGCGGCAAAATGTGTCAGCTTTGTACAAA
TGTGAAGCGGTCAACAAAGTCGGGAGAGGAGAGAGGGTGATCTCCTTCCACGTGACCAGGGGTCTTGAA
ATTACTTTGCAACCTGACATGCAGCCCACTGAGCAGGAGAGCGTGTCTTTGTGGTGCAGTGCAGACAGA
30 TCTACGTTTGGAGAACCTCACATGGTACAAGCTTGGCCACAGCCTCTGCCAATCCATGTGGGAGAGTTG
CCCACACCTGTTTGCAGAAGCTTGGATACTCTTTGGAAATTGAATGCCACCATGTTCTCTAATAGCACA
AATGACATTTTGATCATGGAGCTTAAGAATGCATCCTTGCAGGACCAAGGAGACTATGTCTGCCTTGCT
CAAGACAGGAAGACCAAGAAAAGACATTGCGTGGTCAGGCAGCTCACAGTCTTAGAGCGTGTGGCACCC
ACGATCACAGGAAACCTGGAGAATCAGACGACAAGTATTGGGGAAAGCATCGAAGTCTCATGCACGGCA
35 TCTGGGAATCCCCCTCCACAGATCATGTGGTTTAAAGATAATGAGACCCTTGTAAGAAGACTCAGGCATT
GTATTGAAGGATGGGAACCGGAACCTCACTATCCGCAGAGTGAGGAAGGAGGACGAAGGCCTCTACACC

TGCCAGGCATGCAGTGTCTTGGCTGTGCAAAAGTGGAGGCATTTTTCATAATAGAAGGTGCCCAGGAA
 AAGACGAACTTGGAAATCATTATTCTAGTAGGCACGGCGGTGATTGCCATGTTCTTCTGGCTACTTCTT
 GTCATCATCTTACGGACCGTTAAGCGGGCCAATGGAGGGGAAGTGAAGACAGGCTACTTGTCCATCGTC
 ATGGATCCAGATGAACTCCCATTGGATGAACATTGTGAACGACTGCCTTATGATGCCAGCAAAATGGGAA
 5 TTCCCCAGAGACCGGCTGAAGCTAGGTAAGCCTCTTGGCCGTGGTGCCTTTGGCCAAGTGATTGAAGCA
 GATGCCTTTTGAATTGACAAGACAGCAACTTGCAGGACAGTAGCAGTCAAAATGTTGAAAGAAGGAGCA
 ACACACAGTGAGCATCGAGCTCTCATGTCTGAACTCAAGATCCTCATTCATATTGGTCACCATCTCAAT
 GTGGTCAACCTTCTAGGTGCCTGTACCAAGCCAGGAGGGCCACTCATGGTGATTGTGGAATTCTGCAAA
 TTTGGAAACCTGTCCACTTACCTGAGGAGCAAGAGAAATGAATTTGTCCCCTACAAGACCAAGGGGCA
 10 CGATTCCGTCAAGGGAAAGACTACGTTGGAGCAATCCCTGTGGATCTGAAACGGCGCTTGGACAGCATC
 ACCAGTAGCCAGAGCTCAGCCAGCTCTGGATTTGTGGAGGAGAAGTCCCTCAGTGATGTAGAAGAAGAG
 GAAGCTCCTGAAGATCTGTATAAGGACTTCCTGACCTTGGAGCATCTCATCTGTTACAGCTTCCAAGTG
 GCTAAGGGCATGGAGTTCTTGGCATCGCGAAAGTGTATCCACAGGGACCTGGCGGCACGAAATATCCTC
 TTATCGGAGAAGAACGTGGTTAAAAATCTGTGACTTTGGCTTGGCCCGGGATATTTATAAAGATCCAGAT
 15 TATGTCAGAAAAGGAGATGCTCGCCTCCCTTTGAAATGGATGGCCCCAGAAACAATTTTGGACAGAGTG
 TACACAATCCAGAGTGACGTCTGGTCTTTTGGTGTTTTGTGCTGTGGGAAATATTTTCTTAGGTGCTTCT
 CCATATCCTGGGGTAAAGATTGATGAAGAATTTTGTAGGCGATTGAAAGAAGGAACTAGAATGAGGGCC
 CCTGATTATACTACACCAGAAATGTACCAGACCATGCTGGACTGCTGGCACGGGGAGCCAGTCAGAGA
 CCCACGTTTTTCAGAGTTGGTGAACATTTGGGAAATCTCTTGCAAGCTAATGCTCAGCAGGATGGCAAA
 20 GACTACATTGTTCTTCCGATATCAGAGACTTTGAGCATGGAAGAGGATTCTGGACTCTCTCTGCCTACC
 TCACCTGTTTCTGTATGGAGGAGGAGGAAGTATGTGACCCCAAATTCATTATGACAACACAGCAGGA
 ATCAGTCAGTATCTGCAGAACAGTAAGCGAAAGAGCCGGCCTGTGAGTGTA AAAACATTTGAAGATATC
 CCGTTAGAAGAACCAGAAGTAAAGTAATCCCAGATGACAACCAGACGAGTGGTATGGTTCTTGCC
 TCAGAAGAGCTGAAAACCTTTGGAAGACAGAACC AAATTATCTCCATCTTTTGGTGAATGGTGCCAGC
 25 AAAAGCAGGGAGTCTGTGGCATCTGAAGGCTCAAACCAGACAAGCGGCTACCAGTCCGGATATCACTCC
 GATGACACAGACACCACCGTGTA CTCCAGTGAGGAAGCAGA ACTTTTAAAGCTGATAGAGATTGGAGTG
 CAAACCGGTAGCACAGCCCAGATTCTCCAGCCTGACTCGGGGACCACACTGAGCTCTCCTCCTGTTTAA
 (SEQ ID NO:1), wherein said nucleic acid molecule encodes a human
 KDR protein or biologically active form thereof where at least amino acid
 30 residues selected from the group consisting of Val at position 848, Glu at
 position 498, Ala at position 772, Arg at position 787, Lys at position 835
 and Ser at position 1347 are present in said protein.

2. A purified DNA molecule encoding human KDR
 35 wherein said DNA molecule encodes a protein consisting essentially of
 the amino acid sequence:

MESKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNQSG
 SEQRVEVTECDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVIYVYVQDYRSPFIASVSDQHGVVYI
 TENKNKTVVIPCLGSISNLNVSLCARYPEKRFPDGNRISWDSKKGFTIPSYMISYAGMVFCEAKINDE
 SYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKLVNRD
 5 LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATVG
 ERVRIPAKYLGYPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSEEDTGNYTVILTNPISKEKQSHVVS
 LVVYVPPQIGESLISPVDYQYGTQTTLCTVYAIPPPHHHWYQLEEECANEPSQAVSVTNPYPC
 EWRSEDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTRGPE
 ITLQPDMPTEQESVSLWCTADRSTFENLTWYKLGPPPIHVGEPLTPVCKNLDLWKLNATMFSNST
 10 NDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVRQLTVLERVAPTITGNLENQTTSIGESIEVSCTA
 SGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIIRVRKEDEGLYTCQACSVLGCACVEAFFIIIEGAQE
 KTNLEIIILVGTAVIAMFFWLLLVIIILRTVVKRANGGELKTGYLSIVMDPDELPLDEHCERLPYDASKWE
 FPRDLKLGKPLGRGAFGQVIEADAFGIDKTATCRTVAVKMLKEGATHSEHRALMSELKILIHGHLN
 VVNLGACTKPGGPLMVIVEFCKFGNLSTYLRSKRNEFVPYKTKGARFRQGKDYGAIIPVDLKRRLDSI
 15 TSSQSSASSGFVEEKSLSDVEEEEAPEDLYKDFLTLEHLICYSFQVAKGMEFLASRCKIHRDLAARNIL
 LSEKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWMAPETIFDRVYTIQSDVWSFGVLLWEIFSLGAS
 PYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTMLDCWHGEPSQRPTFSELVEHLGNLLQANAQQDGK
 DYIVLPISETLSMEEDSGLSLPTSPVSCMEEEEVCDPKFHYDNTAGISQYLQNSKRKSRPVSVKTFEDI
 PLEEPEVKVIPDDNQTDGMLVASEELKTLEDRTKLSPSFGGMVPSKSRESVASEGSNQTSQYQSGYHS
 20 DDTDTTVYSSEEAELLKLIIEIGVQTGSTAQILQPDGTTLSPPV, as set forth in a three-
 letter abbreviation in SEQ ID NO:2 and containing amino acid residues
 selected from the group consisting of Val at position 848, Glu at position
 498, Ala at position 772, Arg at position 787, Lys at position 835 and Ser at
 position 1347.

25

3. An expression vector for the expression of a human KDR protein in a recombinant host cell wherein said expression vector comprises the DNA molecule of claim 1.

30

4. An expression vector of claim 3 which is a eukaryotic expression vector.

5. An expression vector of claim 3 which is a prokaryotic expression vector.

35

6. A host cell which expresses a recombinant human KDR protein wherein said host cell contains the expression vector of claim 3.

5 7. A host cell which expresses a recombinant human KDR protein wherein said host cell contains the expression vector of claim 4.

10 8. A host cell which expresses a recombinant human KDR protein wherein said host cell contains the expression vector of claim 5.

15 9. A host cell of claim 6 wherein said human KDR protein is overexpressed from said expression vector.

10. A host cell of claim 7 wherein said human KDR protein is overexpressed from said expression vector.

20 11. A host cell of claim 8 wherein said human KDR protein is overexpressed from said expression vector.

12. A subcellular membrane fraction obtained from the host cell of claim 9 which contains recombinant human KDR protein.

25 13. A subcellular membrane fraction obtained from the host cell of claim 10 which contains recombinant human KDR protein.

30 14. A subcellular membrane fraction obtained from the host cell of claim 11 which contains recombinant human KDR protein.

15. A purified DNA molecule which consists of the nucleotide sequence:

35 ATGGAGAGCAAGGTGCTGCTGGCCGTCGCCCTGTGGCTCTGCGTGGAGACCCGGGCCCTCTGTGGGTT
TGCCTAGTGTCTTCTCTTGATCTGCCCAGGCTCAGCATACAAAAAGACATACTTACAATTAAGGCTAATAC
AACTCTTCAAATTACTTGCAGGGGACAGAGGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGT
GAGCAAAGGGTGGAGGTGACTGAGTGCAGCGATGGCCTCTTCTGTAAGACACTCACAATTCCAAAAGTGA

TCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGGAACTGACTTGGCCTCGGTCAATTTATGTCTA
TGTTCAGATTACAGATCTCCATTTATTGCTTCTGTTAGTGACCAACATGGAGTCGTGTACATTACTGAG
AACAAAAACAAAACGTGGTGATTCCATGTCTCGGGTCCATTTCAAATCTCAACGTGTCACTTTGTGCAA
GATACCCAGAAAAGAGATTTGTTCCCTGATGGTAACAGAATTTCTGGGACAGCAAGAAGGGCTTTACTAT
5 TCCCAGCTACATGATCAGCTATGCTGGCATGGTCTTCTGTGAAGCAAAAATTAATGATGAAAGTTACCAG
TCTATTATGTACATAGTTGTCTGTTGTAGGGTATAGGATTTATGATGTGGTTCCTGAGTCCGTCTCATGGAA
TTGAACTATCTGTTGGAGAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACATAATGTGGGGATTGA
CTTCAACTGGGAATACCCCTTCTTCAAGCATCAGCATAAGAACTTGTAAACCGAGACCTAAAAACCCAG
TCTGGGAGTGAGATGAAGAAATTTTGTAGCACCTTAACATAGATGGTGTAAACCGGAGTGACCAAGGAT
10 TGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAAGACAGCACATTTGTCAGGGTCCATGAAAA
ACCTTTTGTGCTTTTGGAGTGGCATGGAATCTCTGGTGGAAGCCACGGTGGGGGAGCGTGTGAGAATC
CCTGCGAAGTACCTTGGTTACCCACCCCCAGAAATAAAATGGTATAAAAATGGAATACCCCTTGAGTCCA
ATCACACAATTAAAGCGGGGCATGTACTGACGATTATGGAAGTGAGTGAAAGAGACACAGGAAATTACAC
TGTCATCCTTACCAATCCCATTTCAAAGGAGAAGCAGAGCCATGTGGTCTCTCTGGTTGTGTATGTCCCA
15 CCCCAGATTGGTGAGAAATCTCTAATCTCTCTGTGGATTCTTACCAGTACGGCACCACTCAAACGCTGA
CATGTACGGTCTATGCCATTCTCTCCCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGTGCAG
CAACGAGCCCAGCCAAGCTGTCTCAGTGACAAACCCATACCCCTTGTGAAGAATGGAGAAGTGTGGAGGAC
TTCCAGGGAGGAAATAAAATTGAAGTTAATAAAAAATCAATTTGCTCTAATTGAAGGAAAAAACAAACTG
TAAGTACCCCTTGTATCCAAGCGGCAAATGTGTGAGCTTTGTACAAATGTGAAGCGGTCAACAAAGTCGG
20 GAGAGGAGAGAGGGTGATCTCCTTCCACGTGACCAGGGTCTTGAAATTACTTTGCAACCTGACATGCAG
CCCACTGAGCAGGAGAGCGTGTCTTTGTGGTGCAGTGCAGACAGATCTACGTTTGAGAACCTCACATGGT
ACAAGCTTGGCCACAGCCTCTGCCAATCCATGTGGGAGAGTTGCCACACCTGTTTGCAAGAACTTGGA
TACTCTTTGGAATTTGAATGCCACCATGTTCTCTAATAGCACAAATGACATTTTGATCATGGAGCTTAAG
AATGCATCCTTGCAGGACCAAGGAGACTATGTCTGCCTTGCTCAAGACAGGAAGACCAAGAAAAGACATT
25 GCGTGGTCAGGCAGCTCACAGTCTAGAGCGTGTGGCACCCACGATCACAGGAAACCTGGAGAATCAGAC
GACAAGTATTGGGGAAAGCATCGAAGTCTCATGCACGGCATCTGGGAATCCCCCTCCACAGATCATGTGG
TTTAAAGATAATGAGACCCTTGTAGAAGACTCAGGCATTGTATTGAAGGATGGGAACCGGAACCTCACTA
TCCGCAGAGTGAGGAAGGAGGACGAAGGCCTCTACACCTGCCAGGCATGCAGTGTCTTGGCTGTGCAAA
AGTGGAGGCATTTTTTCATAATAGAAGGTGCCAGGAAAAGACGAACCTTGGAATCATTATTCTAGTAGGC
30 ACGGCGGTGATTGCCATGTTCTTCTGGCTACTTCTTGTGCATCATCCTACGGACCGTTAAGCGGGCCAATG
GAGGGGAACCTGAAGACAGGCTACTTGTCCATCGTCATGGATCCAGATGAACTCCCATTTGGATGAACATTG
TGAACGACTGCCTTATGATGCCAGCAAATGGGAATTCCCCAGAGACCGGCTGAAGCTAGGTAAGCCTCTT
GGCCGTGGTGCCTTTGGCCAAGTGATTGAAGCAGATGCCTTTGGAATTGACAAGACAGCAACTTGCAGGA
CAGTAGCAGTCAAATGTTGAAAGAAGGAGCAACACACAGTGAGCATCGAGCTCTCATGTCTGAACTCAA
35 GATCCTCATTCATATTGGTCACCATCTCAATGTGGTCAACCTTCTAGGTGCCGTGTACCAAGCCAGGAGGG
CCACTCATGGTGATTGTGGAATTCTGCAAATTTGGAAACCTGTCCACTTACCTGAGGAGCAAGAGAAATG

AATTTGTCCCCTACAAGACCAAAGGGGCACGATTCCGTCAAGGGAAAGACTACGTTGGAGCAATCCCTGT
 GGATCTGAAACGGCGCTTGGACAGCATCACCAGTAGCCAGAGCTCAGCCAGCTCTGGATTTGTGGAGGAG
 AAGTCCCTCAGTGATGTAGAAGAAGAGGAAGCTCCTGAAGATCTGTATAAGGACTTCCTGACCTTGGAGC
 ATCTCATCTGTTACAGCTTCCAAGTGGCTAAGGGCATGGAGTTCTTGGCATCGCGAAAGTGATCCACAG
 5 GGACCTGGCGGCACGAAATATCCTCTTATCGGAGAAGAACGTGGTTAAAATCTGTGACTTTGGCTTGGCC
 CGGGATATTTATAAAGATCCAGATTATGTCAGAAAAGGAGATGCTCGCCTCCCTTTGAAATGGATGGCCC
 CAGAAACAATTTTTGACAGAGTGACACAATCCAGAGTGACGTCTGGTCTTTTGGTGTTTTGCTGTGGGA
 AATATTTTCCTTAGGTGCTTCTCCATATCCTGGGGTAAAGATTGATGAAGAATTTTGTAGGCGATTGAAA
 GAAGGAAC TAGAATGAGGGCCCCTGATTATACTACACCAGAAATGTACCAGACCATGCTGGACTGCTGGC
 10 ACGGGGAGCCCAGTCAGAGACCCACGTTTTTCAGAGTTGGTGGAACATTTGGGAAATCTCTTGCAAGCTAA
 TGCTCAGCAGGATGGCAAAGACTACATTGTTCTTCCGATATCAGAGACTTTGAGCATGGAAGAGGATTCT
 GGACTCTCTCTGCCTACCTCACCTGTTTCTGTATGGAGGAGGAGGAAGTATGTGACCCCAAATTCATT
 ATGACAACACAGCAGGAATCAGTCAGTATCTGCAGAACAGTAAGCGAAAGAGCCGGCCTGTGAGTGATAA
 AACATTTGAAGATATCCCGTTAGAAGAACCAGAAGTAAAAGTAATCCCAGATGACAACCAGACGGACAGT
 15 GGTATGGTTCTTGCCTCAGAAGAGCTGAAAAC TTTGGAAGACAGAACCAAATTATCTCCATCTTTTGGTG
 GAATGGTGCCAGCAAAGCAGGGAGTCTGTGGCATCTGAAGGCTCAAACCAGACAAGCGGCTACCAGTC
 CGGATATCACTCCGATGACACAGACACCACCGTGTACTCCAGTGAGGAAGCAGAACTTTTAAAGCTGATA
 GAGATTGGAGTGCAAACCGGTAGCACAGCCCAGATTCTCCAGCCTGACTCGGGGACCACACTGAGCTCTC
 CTCCTGTTTAA, disclosed as SEQ ID NO:1.

20

16. A purified human KDR protein which consists of the amino acid sequence

MESKVLVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLWLWPNNQSG
 SEQRVEVTECDGLFCKTLTIQKVIGNDTGAYKCFYRETDLASVIYVYVQDYRSPFIASVSDQHGVVYI
 25 TENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVFCEAKINDE
 SYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLVNCTARTELVNGIDFNWEYPSSKHQHKLVNRD
 LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATVG
 ERVRIPAKYLGYPPPEIKWKNGIPLSNHTIKAGHVLTIMEVSEKDTGNYTVILTNPISKEKQSHVVS
 LVVYVPPQIGEKSLIPVDSYQYGTQTTLCTVYAIPPPHHIHWWQLEEECANEPSQAVSVTNPYPCE
 30 EWRSVEDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTRGPE
 ITLQPDMPTEQESVSLWCTADRSTFENLTWYKLGQPLPIHVGE LPTPVCKNLDTLWKL NATMFSNST
 NDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVVRQLTVLERVAPTITGNLENQTTSIGESIEVSCTA
 SGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTI RVRKEDEGLYTCQACSVLGCAKVEAFFIIEGAQE
 KTNLEIIILVGTAVIAMFFWLLLVIIILRTV KRANGGELKTGYLSIVMDPDELPLDEHCERLPYDASKWE
 35 FPRDRCLKGLPLGRGAFGQVIEADAFGIDKTATCRTVAVKMLKEGATHSEHRAIMSELKILIHGHLN
 VVNLLGACTKPGGPLMVIVEFCKFGNLSTYLRSKRNEFVYPYTKGARFRQGDYVGAIPVDLKRRLDSI

TSSQSSASSGFVEEKSLSDVEEEEAPEDLYKDFTLEHLICYSFQVAKGMEFLASRKC IHRDLAARNIL
 LSEKNVVKICDFGLARDIYKDPDYVRKGDARLPLKWMAPETIFDRVYTIQSDVWSFGVLLWEIFSLGAS
 PYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTMLDCWHGEPSQRPTFSELVEHLGNLLQANAQQDGK
 DYIVLPISETLSMEEDSGLSLPTSPVSCMEEEEVCDPKFHYDNTAGISQYLQNSKRKSRPVSVKTFEDI
 5 PLEEPEVKVIPDDNQTDSGMVLASEELKTLEDRTKLSPSFGCMVPSKSRESVASEGSNQTSQYQSGYHS
 DDTDTTVYSSEEAEELLKLIIEIGVQTGSTAQILQPDSTLSSPPV, as set forth in three

letter abbreviation in SEQ ID NO:2 and containing amino acid residues
 selected from the group consisting of Val at position 848, Glu at position
 498, Ala at position 772, Arg at position 787, Lys at position 835 and Ser at
 10 position 1347.

17. The purified human KDR protein of claim 16 as set
 forth in SEQ ID NO:2.

15 18. A process for the expression of a human KDR protein
 in a recombinant host cell, comprising:

(a) transfecting the expression vector of claim 3 into
 a suitable host cell; and,

20

(b) culturing the host cells of step (a) under
 conditions which allow expression of the human KDR protein from the
 expression vector.

25 19. An expression vector for the expression of a human
 KDR protein in a recombinant host cell wherein said expression vector
 comprises the DNA molecule of claim 15.

30 20. A purified nucleic acid molecule encoding an
 intracellular portion of a human KDR protein which comprises from
 about amino acid 790 to about amino acid 1356 as set forth in SEQ ID NO:
 2, wherein position 848 is a valine residue.

35 21. A purified nucleic acid molecule of claim 20 encoding
 an intracellular portion of a human KDR protein which comprises from
 about amino acid 790 to about amino acid 1356 as set forth in SEQ ID NO:

2, wherein position 772 is an alanine residue, position 787 is an arginine residue, position 835 is a lysine residue, position 848 is a valine residue and position 1347 is a serine residue.

5 22. An expression vector for the expression of a human KDR protein in a recombinant host cell wherein said expression vector comprises the DNA molecule of claim 20.

10 23. An expression vector for the expression of a human KDR protein in a recombinant host cell wherein said expression vector comprises the DNA molecule of claim 21.

15 24. A purified protein fragment which is an intracellular portion of a human KDR protein, comprising from about amino acid 790 to about amino acid 1356 as set forth in SEQ ID NO: 2, wherein position 848 is a valine residue.

20 25. A purified protein fragment of claim 24 which comprises from about amino acid 790 to about amino acid 1356 as set forth in SEQ ID NO: 2, wherein position 772 is an alanine residue, position 787 is an arginine residue, position 835 is a lysine residue, position 848 is a valine residue and position 1347 is a serine residue.

25 26. A purified nucleic acid molecule encoding an soluble KDR fusion protein which comprises from about amino acid 790 to about amino acid 1356 of human KDR as set forth in SEQ ID NO: 2, wherein position 848 is a valine residue.

30 27. A purified nucleic acid molecule of claim 26 wherein said KDR fusion protein comprises from about amino acid 790 to about amino acid 1356 as set forth in SEQ ID NO: 2, position 772 being an alanine residue, position 787 being an arginine residue, position 835 being a lysine residue, position 848 being a valine residue and position 1347 being a serine residue.

35

28. A purified nucleic acid molecule of claim 27 which encodes GST-KDR.

5 29. An expression vector for the expression of a human KDR protein in a recombinant host cell wherein said expression vector comprises the DNA molecule of claim 26.

10 30. An expression vector for the expression of a human KDR protein in a recombinant host cell wherein said expression vector comprises the DNA molecule of claim 27.

15 31. An expression vector for the expression of a human KDR protein in a recombinant host cell wherein said expression vector comprises the DNA molecule of claim 28.

20 32. A purified KDR fusion protein which is characterized by an intracellular portion of a human KDR protein, comprising from about amino acid 790 to about amino acid 1356 as set forth in SEQ ID NO: 2, wherein position 848 is a valine residue.

25 33. A purified KDR fusion protein of claim 32 which comprises from about amino acid 790 to about amino acid 1356 as set forth in SEQ ID NO: 2, wherein position 772 is an alanine residue, position 787 is an arginine residue, position 835 is a lysine residue, position 848 is a valine residue and position 1347 is a serine residue.

34. The purified KDR fusion protein of claim 33 which is GST-KDR.

30 35. A purified nucleic acid molecule encoding an extracellular portion of a human KDR protein which comprises from about amino acid 1 to about amino acid 644 as set forth in SEQ ID NO:2, wherein position 498 is a glutamic acid residue.

36. An expression vector for the expression of a human KDR protein in a recombinant host cell wherein said expression vector comprises the DNA molecule of claim 36.

5 37. A purified protein fragment which is an extracellular portion of a human KDR protein, comprising from about amino acid 1 to about amino acid 790 as set forth in SEQ ID NO: 2, wherein position 498 is a glutamic acid residue, position 772 is an alanine residue and position 787 is an arginine residue.

10 38. An isolated nucleic acid molecule of claim 20 wherein a termination codon is inserted such that the KDR open reading frame terminates at about Tyr 1175.

15 39. An isolated nucleic acid of claim 38 which is contained within a DNA vector, pBlueBacHis2B.

40. The DNA vector of claim 39 which is pBBH-KDR-1.

20 41. A method of selecting a compound which antagonizes human KDR which comprises a biological assay wherein a test compound is added in combination with a KDR protein or protein fragment and a substrate, said substrate being involved in a measurable interaction at a domain of interest within wild-type KDR such that a
25 compound antagonist interacts with said KDR protein, resulting in a measurable decrease in KDR:substrate activity.

42. A method of claim 41 wherein said KDR protein is GST/KDR-1.

30 43. A method of claim 42 wherein said substrate is pEY.

44. A method of selecting a compound which is an agonist of human KDR which comprises a biological assay wherein a
35 test compound is added in combination with a KDR protein or protein fragment and a substrate, said substrate being involved in a measurable

interaction at a domain of interest within wild-type KDR such that a compound antagonist interacts with said KDR protein, resulting in a measurable increase in KDR:substrate activity.

5 45. A method of claim 44 wherein said KDR protein is GST/KDR-1.

 46. A method of claim 45 wherein said substrate is pEY.

10

ATGGAGAGCAAGGTGCTGCTGGCCGTCGCCCTGTGGCTCTGCGTGGAGACCC
GGGCCGCTCTGTGGGTTTGCCTAGTGTTTCTCTTGATCTGCCCAGGCTCAGCA
TACAAAAAGACATACTTACAATTAAGGCTAATACAACCTTTCAAATTACTTGCAG
GGGACAGAGGGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGTGAG
CAAAGGGTGGAGGTGACTGAGTGCAGCGATGGCCTCTTCTGTAAGACACTCAC
AATTCACAAAAGTGATCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGG
AAACTGACTTGGCCTCGGTCAATTTATGTCTATGTTCAAGATTACAGATCTCCATT
TATTGCTTCTGTAGTGACCAACATGGAGTCGTGTACATTACTGAGAACAAAAA
CAAACTGTGGTGATTCCATGTCTCGGGTCCATTTCAAATCTCAACGTGTCACTT
TGTGCAAGATACCCAGAAAAAGAGATTTGTTCTTGATGGTAACAGAATTCCTGG
GACAGCAAGAAGGGCTTTACTATTCCCAGCTACATGATCAGCTATGCTGGCATG
GTCTTCTGTGAAGCAAAAATTAATGATGAAAGTTACCAGTCTATTATGTACATAG
TTGTCGTTGTAGGGTATAGGATTTATGATGTGGTTCTGAGTCCGTCTCATGGAA
TTGAACATATCTGTTGGAGAAAAGCTTGTCTTAAATTGTACAGCAAGAAGTGAAC
TAAATGTGGGGATTGACTTCAACTGGGAATACCCTTCTTCGAAGCATCAGCATA
AGAACTTGTAACCCGAGACCTAAAAACCCAGTCTGGGAGTGAGATGAAGAAA
TTTTTGAGCACCTTAAGTATAGATGGTGTAACCCGGAGTGACCAAGGATTGTAC
ACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTTGTGAG
GGTCCATGAAAAACCTTTTGTGCTTTTGGAAAGTGGCATGGAATCTCTGGTGA
AGCCACGGTGGGGAGCGTGTGAGAATCCCTGCGAAGTACCTTGGTTACCCAC
CCCCAGAAATAAAATGGTATAAAAATGGAATACCCCTTGAGTCCAATCACACAA
TTAAAGCGGGGCATGTACTGACGATTATGGAAGTGAGTGAAAGAGACACAGGA
AATTACACTGTCACTCTTACCAATCCCATTTCAAAGGAGAAGCAGAGCCATGTG
GTCTCTCTGGTTGTGTATGTCCCACCCAGATTGGTGAGAAATCTCTAATCTCTC
CTGTGGATTCTTACCAGTACGGCACCACTCAAACGCTGACATGTACGGTCTATG
CCATTCTCCCCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGTGC
GCCAACGAGCCCAGCCAAGCTGTCTCAGTGACAAACCCATAACCTTGTGAAGA
ATGGAGAAGTGTGGAGGACTTCCAGGGAGGAAATAAAATTGAAGTTAATAAAA
ATCAATTTGCTCTAATTGAAGGAAAAACAAAACCTGTAAGTACCCTTGTTATCCA
AGCGGCAAAATGTGTCAGCTTTGTACAAATGTGAAGCGGTCAACAAAGTCGGGA
GAGGAGAGAGGGTGATCTCCTTCCACGTGACCAGGGGTCTTGAAATTACTTTG
CAACCTGACATGCAGCCCACTGAGCAGGAGAGCGTGTCTTTGTGGTGCATGCT
AGACAGATCTACGTTTGAGAACCTCACATGGTACAAGCTTGGCCACAGCCTCT
GCCAATCCATGTGGGAGAGTTGCCACACCTGTTTGCAAGAACTTGGATACTCT
TTGGAAATTGAATGCCACCATGTTCTCTAATAGCACAAATGACATTTTGATCATG
GAGCTTAAGAATGCATCCTTGACAGGACCAAGGAGACTATGTCTGCCTTGCTCAA
GACAGGAAGACCAAGAAAGACATTGCGTGGTCAGGCAGCTCACAGTCCTAGA
GCGTGTGGCACCCACGATCACAGGAAACCTGGAGAATCAGACGACAAGTATTG
GGGAAAGCATCGAAGTCTCATGCACGGCATCTGGGAATCCCCCTCCACAGATC
ATGTGGTTTAAAGATAATGAGACCCTTGTAGAAGACTCAGGCATTGTATTGAAG
GATGGGAACCGGAACCTCACTATCCGCAGAGTGAGGAAGGAGGACGAAGGCC
TCTACACCTGCCAGGCATGCAGTGTCTTGGCTGTGCAAAAGTGGAGGCATTTT
TCATAATAGAAGGTGCCAGGAAAAGACGAACTTGGAAATCATTATTCTAGTAG
GCACGGCGGTGATTGCCATGTTCTTCTGGCTACTTCTTGTGTCATCATCCTACGGA
CCGTTAAGCGGGCCAATGGAGGGGAACTGAAGACAGGGTACCTGTCCATCGT
CATGGACCCAGATGAACTCCCATTTGGATGAACATTGTGAACGACTGCCTTATGA
TGCCAGCAAATGGGAATTCCCAGAGACCGGCTGAAGCTAGGTAAGCCTCTTG
GCCGTGGTGCCTTTGGCCAAGTGATTGAAGCAGATGCCTTTGGAATTGACAAG
ACAGCAACTTGCAGGACAGTAGCAGTCAAATGTTGAAAGAAGGAGCAACACA
CAGTGAGCATCGAGCTCTCATGTCTGAACTCAAGATCCTCATTTCATATTGGTCA
CCATCTCAATGTGGTCAACCTTCTAGGTGCCTGTACCAAGCCAGGAGGGCCAC
TCATGGTGATTGTGGAATTCTGCAAAATTTGGAAACCTGTCCACTTACCTGAGGA
GCAAGAGAAATGAATTTGTCCCCTACAAGACCAAAGGGGCACGATTCCGTCAA
GGGAAAGACTACGTTGGAGCAATCCCTGTGGATCTGAAACGGCGCTTGGACAG

FIGURE 1B

CATCACCAGTAGCCAGAGCTCAGCCAGCTCTGGATTTGTGGAGGAGAAGTCCC
TCAGTGATGTAGAAGAAGAGGAAGCTCCTGAAGATCTGTATAAGGACTTCCTG
ACCTTGGAGCATCTCATCTGTTACAGCTTCCAAGTGGCTAAGGGCATGGAGTTC
TTGGCATCGCGAAAGTGTATCCACAGGGACCTGGCGGCACGAAATATCCTCTT
ATCGGAGAAGAACGTGGTTAAAATCTGTGACTTTGGCTTGGCCCCGGGATATTTA
TAAAGATCCAGATTATGTCAGAAAAGGAGATGCTCGCCTCCCTTTGAAATGGAT
GGCCCCAGAAACAATTTTTGACAGAGTGTACACAATCCAGAGTGACGTCTGGT
CTTTTGGTGTGTTTGTCTGTGGGAAATATTTTCCTTAGGTGCTTCTCCATATCCTGG
GGTAAAGATTGATGAAGAATTTTGTAGGCGATTGAAAGAAGGAACTAGAATGA
GGGCCCCTGATTATACTACACCAGAAATGTACCAGACCATGCTGGACTGCTGG
CACGGGGAGCCCAGTCAGAGACCCACGTTTTTCAGAGTTGGTGGAACATTTGGG
AAATCTCTTGCAAGCTAATGCTCAGCAGGATGGCAAAGACTACATTGTTCTTCC
GATATCAGAGACTTTGAGCATGGAAGAGGATTCTGGACTCTCTCTGCCTACCTC
ACCTGTTTCCTGTATGGAGGAGGAGGAAGTATGTGACCCCAAATTCATTATGA
CAACACAGCAGGAATCAGTCAGTATCTGCAGAACAGTAAGCGAAAGAGCCGGC
CTGTGAGTGTAAAAACATTTGAAGATATCCCGTTAGAAGAACCAGAAGTAAAAG
TAATCCCAGATGACAACCAGACGGACAGTGGTATGGTTCTTGCCTCAGAAGAG
CTGAAAACCTTTGGAAGACAGAACCAAATTATCTCCATCTTTTGGTGGAAATGGTG
CCCAGCAAAAGCAGGGAGTCTGTGGCATCTGAAGGCTCAAACCAGACAAGCG
GCTACCAGTCCGGATATCACTCCGATGACACAGACACCACCGTGTACTCCAGT
GAGGAAGCAGAACTTTTAAAGCTGATAGAGATTGGAGTGCAAACCGGTAGCAC
AGCCCAGATTCTCCAGCCTGACTCGGGGACCACACTGAGCTCTCCTCCTGTTTA
A

MESKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQR
DLDWLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVI GNDTGAYKCFYRETDLAS
VIYVYVQDYRSPFIASVSDQHGVVYTENKNKTVVIPCLGSISNLNVSLCARYPEKR
FVPDGNRISWDSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYTVVVVGYRIYDV
VLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKLVNRDLKTQS
GSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGM
ESLVEATVGERVRIPAKYLGYPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDT
GNYTVILTNPISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYGTTQTTLCTVYAI
PPHHIHWYWQLEEECANEPSQAVSVTNPYPCEEWRSVEDFQGGNKIEVNKNQFA
LIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTRGPEITLQPDMP
TEQESVSLWCTADRSTFENLTWYKLGPOPLPIHVGE LPTPVCKNLDTLWKL NATM
FSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVRQLTVLERVAPTTGN
LENQTTSIGESIEVSCTASGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIRVRK
EDEGLYTCQACSVLGCAKVEAFFIIEGAQEKTNLEIII L VGTAVIAMFFWLLLVIILRT
VKRANGGELKTGYLSIVMDPDELPLDEHCERLPYDASKWEFPRDRLKLGKPLGRG
AFGQVIEADAFGIDKTATCRTVAVKMLKEGATHSEHRALMSELKILIHIGHHLNVV
NLLGACTKPGGPLMVIVFECKFGNLSTYLRSKRNEFVYPYKTKGARFRQGDYVG
AIPVDLKRRLDSITSSQSSASSGFVEEKSLSDVEEEEAPEDLYKDFTLEHLICYSFQ
VAKGMEFLASRKCIHRDLAARNILLSEKNVVKICDFGLARDIYKDPDYVRKGDAR
LPLKWMAPETIFDRVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRRLKEGT
RMRAPDYTTPEMYQTMLDCWHGEP SQRPTFSELVEHLGNLLQANAQQDGDYTVL
PISETLSMEEDSGLSLPTSPVSCMEEEEVCDPKFHYDNTAGISQYLQNSKRKSRPVS
VKTFEDIPLLEEPEVKVIPDDNQTDSGMVLASEELKTLEDRTKLSPSF GGMVPSKSRE
SVASEGSNQTSQGYQSGYHSDDTDTTVYSSEEAE LLKLIIEIGVQTGSTAQILQPDSGT
TLSSPPV

FIGURE 3A

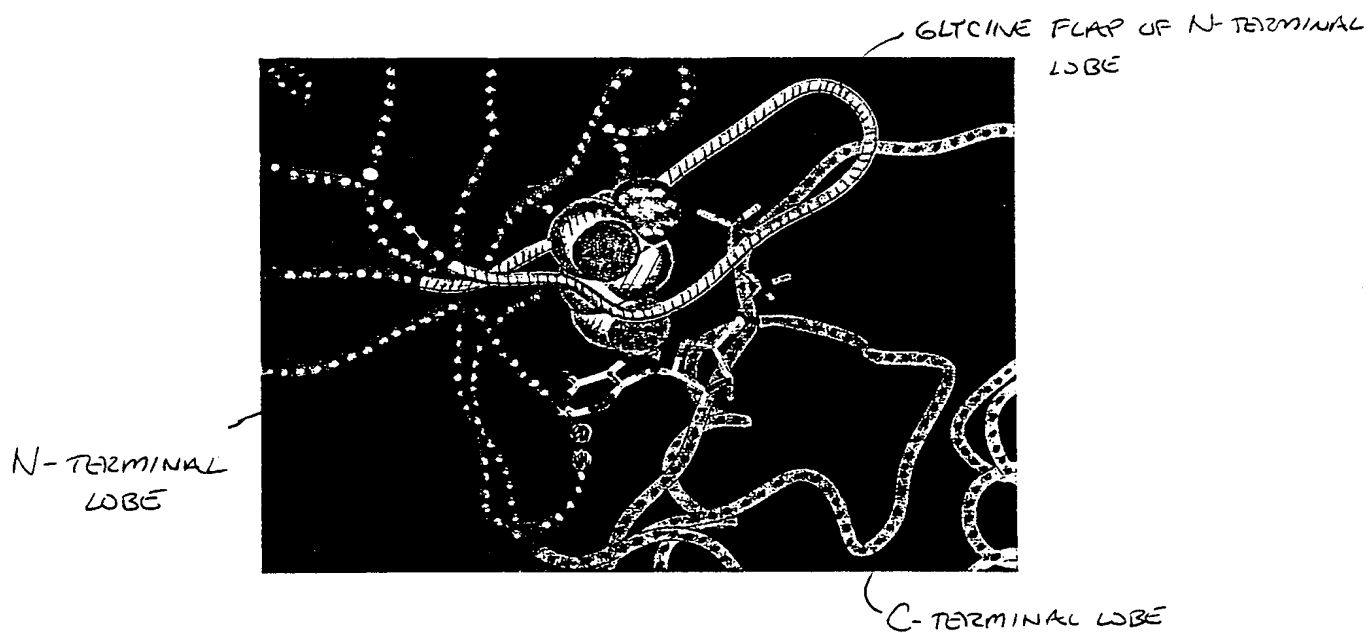


FIGURE 3B

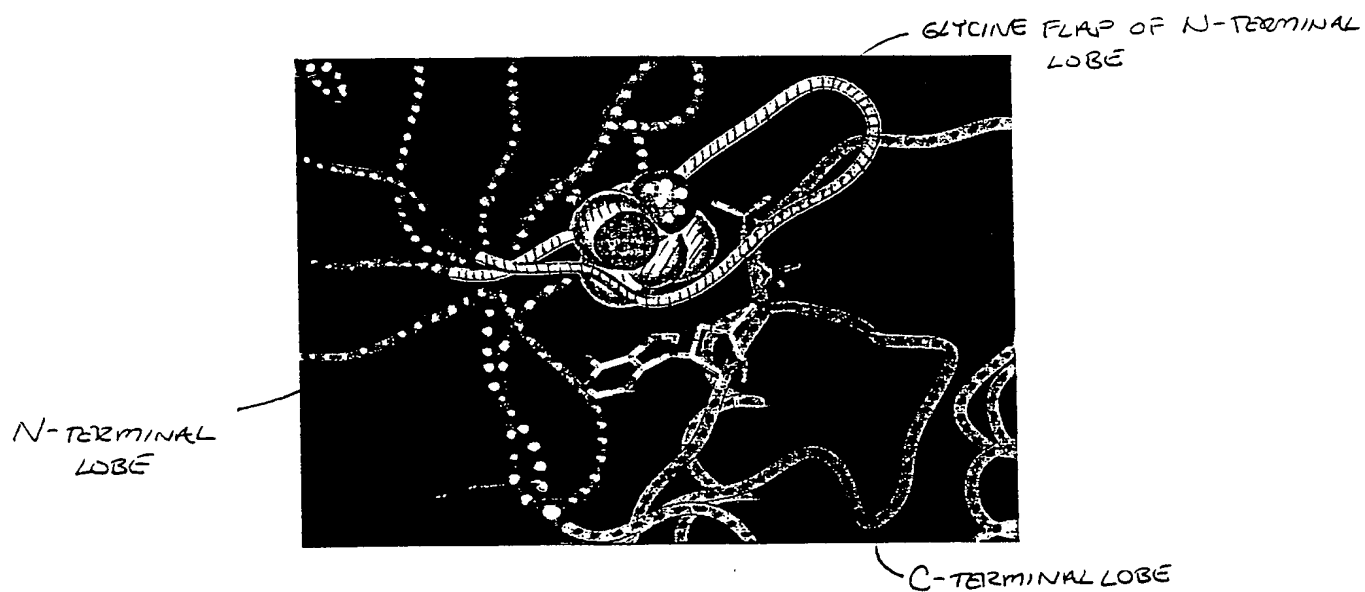


FIGURE 4A

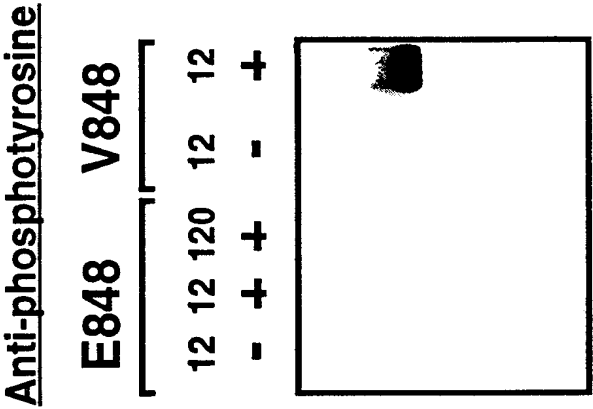
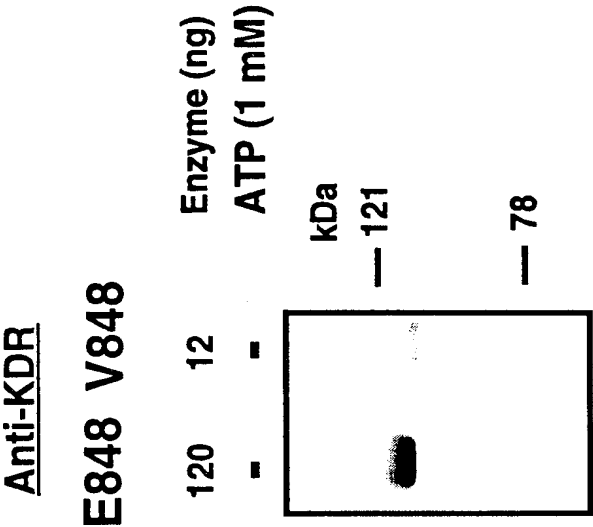


FIGURE 4B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/12569

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/194, 69.1, 252.3, 320.1, 325, 361; 436/501; 530/350; 536/23.2, 23.5.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; STN FILES - Medline, Caplus, Wpids, Biosis, Biotechds, Scisearch. Search terms included : receptor tyrosine kinase (RTK), human KDR and growth factor?.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TERMAN B. I. Identification of a new Endothelial Cell Growth Factor Receptor Tyrosine Kinase. Oncogene 1991. Vol 6. pages 1677-1683. See Table 1 and Figures 1-3.	1-40
Y	TERMAN B. I. Identification of the KDR Tyrosine Kinase as a Receptor for Vascular Endothelial Cell growth Factor. Biochem. Biophys. Res. Com. 30 September 1992. Vol. 187. No. 3. pages 1579-1586.	1-40
A, P	US 5,766,860 A (TERMAN ET AL.) 16 June 1998, see Figure 7 (A-M), claim 1 in column 43 & 44.	41-46

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 14 AUGUST 1998	Date of mailing of the international search report 08 SEP 1998 <i>TEKHAND SAIDHA</i>
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/12569

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C12N 9/12, 1/20, 15/00; G01N 33/53; C07K 1/00; C07H 21/04.

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/194, 69.1, 252.3, 320.1, 325, 361; 436/501; 530/350; 536/23.2, 23.5.